BOOKS RECEIVED

MEDICAL EDUCATION, A Comparative Study, by Abraham Flexner, 1925; 334 pages, size 6 × 9¼, cloth, $2.50. The Macmillan Company, New York. "The present volume attempts to make a comparative study of medical education in certain European countries and America against the background afforded by the general education and social systems of the respective countries."—Preface.

THE EARLY EMBRYOLOGY OF THE CHICK, by Bradley M. Patten, second edition, 1925; 178 pages, illustrated in black and color, 213 figures, size 6 × 9¼, cloth, P. Blakiston's Son & Co., Philadelphia. "The primary purpose of this book is to provide a brief and simple text for the beginning student. The addition of any considerable amount of new material would tend to defeat this purpose."—Preface.

SMELL, TASTE, AND ALLIED SENSES IN THE VERTEBRATES, by G. H. Parker, 1922, Monographs on Experimental Biology Series; 192 pages, size 5½ × 8, cloth, $2.50, J. B. Lippincott Company, Philadelphia. "Sense organs are the means of approach to the human mind. The deaf and the blind show how serious is the loss of even a single set of these organs."—Preface.

DEVELOPMENTAL ANATOMY, A Text Book and Laboratory Manual of Embryology, by Leslie B. Arey, Professor of Anatomy at the Northwestern University Medical School, Chicago, 1924; 434 pages, cloth, $5.50, 419 illustrations, many in color, W. B. Saunders Company, Philadelphia and London. "For the use of medical students and others whose interests center primarily on man and mammals—emphasizing of structure rather than functional aspects of embryology."—Preface.

BRAIN ABSCESS, ITS SURGICAL PATHOLOGY AND OPERATIVE TECHNIQUE, by Wells P. Eagleton, New York: The Macmillan Co., 1922. The text includes a description of the microscopical lesions of brain abscess followed by the physiological and mechanical conditions which must be dealt with surgically and a description of the technic best adapted to meet them. The work is based primarily on the author's personal experience and its value is greatly enhanced by full citation and analysis of cases in the recent literature. It is, in fact, an encyclopaedia of the surgical pathology and operative treatment of brain abscess.

DIE KRETINISCHE ENTARTUNG, by Dr. Ernst Finkbeiner; 432 pages, illustrated. Berlin: Springer, 1923. This monograph is a compilation and bibliography of cretinism. The references number 647, about 2 per cent of which are to American workers. The author defines cretinism as an endemic degeneration form of European people. Heredity is considered as its main causal agents. Hypothyreosis is an accompaniment of the general degeneration, not a cause. The analysis of the data collected from other workers and that obtained from a few protocols and skeletons of cretins is based on anthropological measurements of the long bones, hands and feet, and some skull measure-

ments. The deviations of these structures from the normal are carefully described, both as such and in relation to the body form as a whole. From the descriptive point of view, the monograph is valuable. From the point of view of the ideas expressed, it is stimulative of thought, but hardly convincing. In addition to the anthropological discussion there is given considerable space to the ergology of the cretin.—F. S. HAMMETT.

REPORT ON SECOND INTERNATIONAL CONGRESS OF MILITARY MEDICINE AND PHARMACY, Rome, May–June, 1923, by Commander William Seaman Bainbridge, M.C., United States Naval Reserve Forces, Member of Permanent Committee, Delegate from the United States. Reprinted from *The Military Surgeon*, December, 1924; January and February, 1924, 148 pages, 34 half-tone illustrations, size $5\frac{1}{2} \times 8\frac{3}{4}$, cloth, Washington, D. C. "The historical value of these reports renders the present volume an important contribution to the records of the World War. The report of the First International Congress appeared in the U. S. Naval Medical Bulletin for December, 1922."—Foreword.

STUDENTS' GUIDE TO OPERATIVE SURGERY, by Alfred T. Bazin, D.S.O., M.D., Assistant Professor of Surgery, McGill University, 1923; 126 pages, size $7\frac{3}{4} \times 4\frac{3}{4}$, cloth, $2.50, Renou Publishing Company, Montreal, Canada. "This little book is the evolution of the experience of recent years in directing a practical class of undergraduate students through a series of operations upon the cadaver. Its small size enables the student to use it as he works. It is his guide, not only as he works, but as he reads. There is a chapter on the Organization of the Class and a Schedule of the Operations, which may be of service to other teachers of the subject."—Preface.

A CHAPTER IN AMERICAN EDUCATION, Rensselaer Polytechnic Institute, 1824–1924, by Ray Palmer Baker, Ph.D., 1924; 170 pages, size $5 \times 7\frac{1}{4}$, Charles Scribner's Sons, New York. The centennial celebration, Rensselaer Polytechnic Institute, oldest college of science and engineering in any English-speaking country, was celebrated October 3 and 4, 1924. The author calls attention to the facts that the Institute introduced field work and laboratory practice in the United States, and that its graduates were pioneers in zoölogy, laying the foundations of many university departments and government bureaus, and adds: "Though the institution as a whole never materialized for lack of funds; though Agassiz, Cook, Emmons, Hall, Mitchell, and Norton were evidently the only members of the group who actually delivered lectures; and though the Dudley Observatory, established by Pierce, Mitchell, and Norton, is to-day the only monument of this attempt to found a national university with generous provision for research, the proposal is illustrative of the influence which Rensselaer, through its curriculae or its alumni, has exerted throughout America.

RHUS DERMATITIS, From Rhus Toxicodendron, Radicans and Diversiloba (Poison Ivy), Its pathology and chemotherapy, by James B. McNair, 1923; 298 pages, 15 figures and 3 halftone plates, size 6×9, cloth, $1.50, The University of Chicago Press, Chicago. In this book the author has made a comprehensive study of the poison in the plant, its origin and occurrence, its seasonal variations, and its transmission from plant to person. He discusses the chemistry of the poison, the pathology of the resultant disease during its different stages, the after-effects and recurrence, and, finally, treatment and remedies presented from a scientific point of view.

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REGRESSIVE STRUCTURES AND THE LYMPHOCYTE

THE PLASMA CELL; ITS ORIGIN AND DEVELOPMENT. A STUDY OF THE MAMMALIAN NICTITATING MEMBRANE

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ONE PLATE (SIX FIGURES)

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INTRODUCTION

The apparent degeneration, regression, and change of function of an organ were made the subject of a paper by Anton Dohrn in 1875, in which he discussed the 'endocrine organs' from a genetic point of view. In 1891, Stöhr suggested that most of the lymphatic tissue occurring outside of the lymph and blood vessels and the lymph nodes was the agent of resorption processes. In support of this view, he mentioned groups of these cells occurring in organs which appear to be undergoing regression and degeneration, such as the pronephros of lower vertebrates, the gills of anura, the thymus and the vermiform appendix. He also suggested the gland of the nictitating membrane as a place where these

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degenerative changes may be taking place. In 1912, Kingsbury added the amphibian tonsils and the tubal tonsils of birds to the above list, and, in 1913, Jolly mentioned a group of so-called 'lympho-epithelial organs' in which there appeared masses of lymphocytes which seemed to be associated with regressive changes and changes in function in these organs. The organs mentioned are the Peyer's patches, the tonsils, the esophageal lymphoid organs of birds, the lymphoid papillae of anal glands, the thymus of teleosts, the bursa Fabricii of birds, and the thymus of most vertebrates. In 1915, Kingsbury discussed this peculiar relationship between apparently regressive organs and lymphoid tissue, and suggested that the presence of lymphocytes in these places might be part of 'a fundamental mesenchymal reaction' toward a tissue which has altered its normal physiological function.

Leo Loeb, who for more than twenty years has been working with tissue transplants and hence has been in a position to study carefully the relationship between different groups of tissues, states the following, which was also referred to by Kingsbury in 1915:

> A certain metabolic activity on the part of parenchyma of various organs determines the attitude of lymphocytes and of fibroblasts towards the parenchyma. If the activity is normal, lymphocytes do not, or only to a slight extent, enter the parenchyma. The connective tissue is held in a definite state of activity (p. 728) . . . . While the vitality of the tissues has not been essentially impaired after homoiotransplantation, and they may even grow, and the metabolic changes they underwent in the different chemical environment did not therefore markedly interfere with their power to live or even propagate, these metabolic changes lead to a new condition in the host tissue, which secondarily brings about the destruction of the transplanted piece, namely, these metabolic changes cause (a) an increased activity on the part of small mononuclear cells (probably lymphocytes) and (b) a destructive activity on the part of the connective tissue of the host. The mononuclear cells collect around the transplanted tissue, penetrate into it and destroy its cells . . . . We may conclude therefore that the metabolic changes taking place in tissues after homoiotransplantation stimulate the activity of the lymphocytes and cause the altered function of the fibroblasts which now produce

fibrous tissue in large quantity. . . . . The activity of lymphocytes and of connective tissue is to some extent independent of each other; while in many cases the two act together, in some cases the one prevails, in other cases the other (p. 727). Connective tissue and lymphocytes may therefore be regarded as organs of attack, lying quiescent under ordinary conditions, but exerting their efforts as soon as within given limits certain pathologic changes take place (p. 728).

The results obtained by Jas. B. Murphy ('21) from studies of tissue grafting have been similar to those of Loeb.

Hence, while to-day the function of the lymphocyte is not definitely known, the work of Loeb, Murphy, Bunting, and others has suggested strongly that it is concerned with the removal of tissues, either foreign or local, which have become abnormal in their activity, and with the affixation of toxins which this metabolic change has been instrumental in forming or toxins which have been produced by bacteria. It is thus easier to understand the association of the lymphocyte with the regressive changes which are apparently going on in the organs mentioned earlier in this paper.

PRESENT INVESTIGATION

In the list of the organs mentioned by Stöhr, a question mark is placed after the glands of the nictitating membrane (the plica semilunaris in man), and at the suggestion of Dr. B. F. Kingsbury I have studied these glands in the cat and in the rabbit with the object of ascertaining whether or not these glands are undergoing regression, and what relation, if any, exists between this regression and any lymphatic tissue which may be present.

MATERIAL AND METHODS

The materials used in this investigation consisted of ten series of nictitating membranes of cats ranging in ages from five days before birth to twenty years after, besides material which was not made up into series, and four series of nictitating membranes taken from rabbits whose ages ranged from forty-five days to two years. Similar material was taken

from other rabbits, but was not made up into series. The fixers employed were 10 per cent formalin, Helly's, Zenker's, and Bensley's. The stains employed were hematoxylin and eosin, alcoholic eosin and methylene blue, Mallory's connective-tissue stain, Downey's special stain for the demonstration of fibers, iron hematoxylin, and hematoxylin and picro-fuchsin. The best stains for the study of plasma cells were found to be Mallory's connective-tissue stain, iron hematoxylin, and alcoholic eosin and methylene blue.

THE NICTITATING MEMBRANE

The nictitating membrane is an anchor- or shield-shaped structure situated at the inner or medial angle of the eye. It has two surfaces, an inner, or orbital, and an outer, the outer one being convex and the inner, concave. The membrane is so shaped that the base or medial extremity lies beneath the medial and dorsomedial surface of the eyeball, so that any pressure on the eyeball will cause the membrane to slide forward over the medial anterior surface of the bulb. A fold of typical conjunctival epithelium is reflected over the outer two-thirds of the membrane except at the tip, where the epithelium is stratified squamous and, in most cases, heavily pigmented. The epithelium rests upon a layer of white fibrous connective tissue. In the center of the membrane is an anchor-shaped hyalin cartilage which extends from almost the extreme outer or lateral edge of the membrane to within a short distance of the inner or medial tip or base. At the base is a large pad of fat. In the cat the membrane measures about $1\frac{3}{4}$ to 2 cm. from the outer edge to the base and about $1\frac{1}{2}$ cm. in width at its outer or lateral edge. In the rabbit it is smaller, being about $1\frac{1}{8}$ to $1\frac{1}{2}$ cm. from outer edge to base and about $1\frac{3}{8}$ cm. in width at its outer or lateral edge.

THE GLANDS OF THE NICTITATING MEMBRANE

The glands of the nictitating membrane are: 1) that of the nictitating membrane proper or true gland of the nicti-

tating membrane, which is located on both sides of and at the base of the cartilage, and, 2) the harderian gland which is situated at the outer surface of the tip of the base of the membrane in close relation to the true gland. It is surrounded by a thin investment of connective tissue.

The structure of the gland is similar to that of the lacrimal gland; i.e., it is of the tubulo-acinar type. It really consists of a number of separate glands, twelve to eighteen in the cat and fifteen to thirty in the rabbit, which open to the surface on the middle of the orbital side in the cat and on the outer third of the orbital surface of the membrane in the rabbit.

The structure of the harderian gland is of the compound tubulo-alveolar type. The gland is drained by a single duct which opens on the orbital side of the membrane in the area into which the ducts from the true gland open.

Both glands are present in the rabbit, but I have not found the harderian gland in any of the cats which I have examined, the only gland present being that of the nictitating membrane proper. The term 'harderian gland' has been rather loosely used in the past, some applying it to the true gland of the nictitating membrane.

LYMPHATIC TISSUE; OBSERVATIONS AND SUMMARY

In the material obtained from the animals used in this investigation, four features in particular come up for consideration, namely, 1) the variation of the amount and extent of lymphatic tissue present with the age of the animal; 2) the source of the lymphocytes present; 3) the relation of this lymphatic tissue to the glandular structures present; 4) the occurrence and origin of plasma cells. It should be mentioned here that the following discussion refers to the true gland of the nictitating membrane only, and not to the harderian gland, which was found to show no regressive changes. Some lymphocytes were found situated close to the excretory duct of the harderian gland, but these interfered in no discernible manner with the normal function of that organ.

1. The variation of the amount and extent of the lymphatic tissue present with the age of the animal

Lymphocytes were absent from material taken five days before and five days after birth. At ten days after birth a few were found scattered diffusely in the connective tissue underlying the conjunctival epithelium on the orbital side of the membrane and between the gland lobules. From this time on, up to and including the middle-aged adult animals, the increase in the number of lymphocytes is rapid and considerable. A 12-cm. animal shows the presence of three distinct nodules besides some diffuse lymphatic tissue; a half-grown animal shows thirty nodules present, and the young and middle-aged adults show a considerable increase over the half-grown animal in the number of lymphocytes present.

With the onset of old age a decrease in the lymphatic tissue is demonstrated by the examination of material from an eight-year-old cat. Material from a cat of fifteen years shows a still further decrease in this tissue, the condition present being very similar to that in a cat of twenty years.

The lymphatic tissue present in the nictitating membrane is thus seen to begin its development some time between the fifth and tenth days after birth and to increase rapidly in amount and extent with the age of the animal up to and including the middle-aged adult; i.e., an animal in the prime of life. With the passing of middle age and the onset of senility a gradual decrease in the number of lymphocytes occurs. This decrease, however, does not proceed to an extent where lymphocytes are very few in number for, even in the oldest animals examined, a certain amount of lymphatic tissue still remained.

2. The source of the lymphocytes present

The development of lymphocytes from the connective tissue of the nictitating membrane and the increase in the number of lymphocytes by the mitotic division of those developing from this tissue is so conspicuous and the evidence of their migra-

tion from the blood and lymph vessels so slight that one is forced to the conclusion that most, if not all, of the lymphocytes present come from the connective-tissue stroma in the vicinity of the gland involved. It is not my purpose to discuss all the possible sources from which lymphocytes may arise or the theories of different workers supporting this or that particular point of view. Suffice it to say that enough work has been done on the origin and development of the lymphocyte to indicate that it may arise from a number of different sources and that connective tissue is one of those sources. It appears to me that they develop directly from fibroblasts. The recent publications of Maximow ('22, '23) state that such is not the case; that lymphocytes may develop from reticulum cells of connective tissue, but not from fibroblasts; that both fibroblasts and reticulum cells are so similar in ordinary preparations that they may be easily mistaken for one another; that they are easily differentiated if studied in tissue cultures. I am not in a position to dispute this point, for it may be that what I have called fibroblasts are really reticulum cells. The point I desire to stress, however, is not whether lymphocytes come from fibroblasts or from reticulum cells, but that *loose connective tissue retains its embryonic prospective developmental potencies throughout life and is a potential source of lymphocytes*, the production of the lymphocytes depending upon the disturbance of the epitheliomesenchymal balance. The study of the lymphoid tissue in this membrane is an excellent demonstration of this fact.

3. The relation of the glandular structures to the lymphatic tissue

The relation of the gland of the nictitating membrane to the lymphocyte is a peculiar and important one, so it is best to discuss it in some detail.

From the beginning of their development, the lymphocytes are for the most part located on the orbital side of the nictitating membrane in the area occupied by the gland ducts.

The nodules, in the great majority of cases, are found on the side of the duct nearest to the surface of the membrane. The epithelium of the duct next to the nodule becomes hypertrophied (fig. 1), so that a growth into the lumen of the duct is formed (fig. 2). This invagination is, in some instances, so great that the lumen is nearly obliterated. Some of the cells of the duct epithelium may form a cytoreticulum, within the meshes of which are crowded lymphocytes. Other epithelial cells are desquamated into the duct lumen which also contains lymphocytes which have passed through the duct wall. This debris—disintegrating lymphocytes and epithelial cells mainly—in many cases causes a complete closing of the duct, so that many cystic ducts, some of them of a large diameter, are formed. The conjunctival epithelium in the area of the nodules is also invaded by the lymphocytes and presents an appearance very similar to that of the epithelium covering the palatine tonsils; i.e., the continuity of the epithelium is broken and lymphocytes are passing out upon the surface. The gland acini in many places are also invaded by lymphocytes, the secretory cells showing all stages of karyorrhexis, karyolysis, and cytolysis.

Closely associated with this activity of the lymphocytes is that of the fibrous connective tissue which seems to succeed the lymphocytic reaction. Areas, which in young animals show little invasion by lymphocytes, in older animals show an intensive infiltration by these cells, and these areas in still older animals show the replacement of the lymphatic tissue by denser fibrous connective tissue. This is true not only of areas occupied by gland acini, but also of those occupied by the ducts, the number of which in older animals is greatly reduced as compared to the number present in young specimens.

It is important to note that degenerative changes taking place in the gland are not necessarily dependent upon the presence of lymphocytes. In fact, many acini are found which show all stages of degeneration and yet are free from lymphocytes. This is particularly well shown in the case of

the older animals where entire lobules show deterioration, there being few or no lymphocytes in the field.

The relationship of the lymphatic tissue present is thus primarily one of nodule to duct, most of the lymphocytes being limited to the region of the excretory ducts. The gland acini are also invaded by the lymphocytes, but the effect of this invasion is secondary in both extent and importance to the effect of the lymphocytes on the ducts.

Summary

The examination of the material obtained from both the cat and the rabbit convinces the observer that the true gland of the nictitating membrane is a regressive structure and that this regression bears a close relation to the lymphoid tissue present.

The primary cause of this regression cannot be definitely stated. It may be that the metabolic activity of the gland has altered sufficiently to disturb the normal balance between the epithelial and stromal constituents; the blood supply of the organ may have become insufficient (the congested appearance of the vessels of the membrane make this a possibility); or some other unknown factors may be instrumental in the alteration of function and deterioration of this organ. It is certain, however, that some change in the gland has occurred which has brought about a reaction which contributes materially to the removal of the gland. This reaction is characterized by the invasion and obliteration of many of the ducts and acini of the gland. The choking of the ducts causes a backing up of the glandular secretion, the formation of cystic ducts, and the degeneration of those acini pouring their secretion into the particular duct affected.

The invasion of the above areas by the lymphocytes is followed by a more fibrous growth which eventually replaces those areas of degeneration. Some few degenerated areas were found to be replaced by adipose tissue instead of fibrous connective tissue.

Hence, while the presence of the lymphocyte in the nictitating membrane bears a close relation to the regressive changes which are taking place in the glands of this organ, it is not the primary or fundamental factor or cause of this regression, but may be regarded as the expression of a reaction toward a tissue which has altered its normal physiological function. Whether the lymphocyte actively invades and destroys, whether it neutralizes toxins formed by the autolysis of the dying cells, whether it aids in that autolysis, or whether it has some other significance there cannot be stated.

PLASMA CELLS

Historical

Of all cell types, the origin and morphology of the plasma cell have, perhaps, been the most disputed. When Waldeyer in 1875 first introduced the term 'Plasmazellen' he applied it to a group of connective-tissue cells which were particularly rich in cytoplasm as compared to those cells of the connective tissue in which the cytoplasm was scanty. This group included a variety of cell types, as later investigations (Unna, Waldeyer, and others) showed, among them being Ehrlich's mast cells, the cells of the corpus luteum, and others.

In 1875, Unna, who had been working with pathological skin conditions, applied the term 'Plasmazellen' to a cell which he had found in granulation tissue and which he had discovered by the employment of special staining methods. The justification of applying this term to this cell was recognized by Waldeyer in 1895. Since, by this time, most of the cells included in Waldeyer's group had become known by other distinctive names, the term 'Plasmazellen' became reserved for the cell described by Unna as follows: "Plasma cells are to be defined as essentially unilaterally hypertrophied connective tissue cells in which the granular constituent of the cytoplasm is increased to a maximum extent." By granular Unna meant amorphogranular, and not the granules of Ehrlich or cellular inclusions. Very little atten-

tion was paid to the nucleus, the characteristic and important thing required in identifying this cell being the observance of what Unna termed 'granuloplasm.' These cells had no definite shape, originated directly from the connective tissue, and divided amitotically. The cytoplasm of these cells (i.e., the granuloplasm) was strongly basic.

The above definition by Unna aroused much opposition, both his morphological definition and his interpretation of the origin of the cell being disputed. Probably the leading figure of the opposition was Marschalkó. Marschalkó, after a study of both the nucleus and the cytoplasm of this cell, selected from Unna's group a particular type of cell which was definite in form and which he claimed was the true mature plasma cell. This cell possessed, in addition to the granuloplasm and tinctorial properties described by Unna, a round eccentric nucleus, the chromatin material of which was grouped into several dense blocks, the bases of which rested on the nuclear membrane. On one side of the nucleus there existed a spot in the cytoplasm in which the granuloplasm appeared less dense, thus giving the appearance of a small light area. This area has been shown by later investigators (Maximow, Wallgren, and others) to be the centrosphere. The cell outline was round or oval in shape and the cell originated from a hematogenous lymphocyte and not from connective tissue, as claimed by Unna. Unna, still believing his ideas in regard to this cell to be the correct ones, replied by reaffirming his former assertions and described Marschalkó's cell as a plasma cell, but as an atrophic one, the light area being due to a washing out of the granuloplasm. The small cells described by Marschalkó as intermediate stages in the transformation of hematogenous lymphocytes into plasma cells, Unna explains as plasma cells which have lost most of their amorphogranular cytoplasm by fragmentation and washing-out processes. It might be said, however, that since then the type of cell described by Marschalkó has been shown to be not atrophic and that it is generally accepted at the present time as the typical mature plasma cell.

Other investigators offered an abundance of evidence on the question; however, most of it dealing with the origin of the plasma cells was conflicting. With the increase in the number of investigations, the workers in this field gradually became divided into three main groups—those insisting on the, *a*) histogenous origin, *b*) hematogenous origin, *c*) both a hematogenous and a histogenous origin. Among the first group may be mentioned Unna, Pappenheim, Herbert, Leo Ehrlich, Porcile, Veratti, Schaeffer, Rheindorf, Weil, and Joannovics. These investigators, with the exception of Unna, believed that the plasma cells first go through a lymphocytic stage and do not originate directly from the connective tissue. Some of those supporting the hematogenous theory were Schottlander, Krompecher, Marschalkó, Baumgarten, Dominici, Justi, Maximow, Nissl, Wallgren, and Dubieuil. The dual origin was supported by Joannovics ('99). Joannovics later ('09) gave his support to the histogenous origin. Schlessinger, Weidenreich, and Downey were other workers believing in both a hematogenous and a histogenous origin.

Most of the above work was done with pathological material, but the work of Downey ('10), in addition to being very complete, was done with normal animals, both warm- and cold-blooded. I wish to quote from his paper:

(P. 309.) Plasma cells are differentiated from all types of lymphoid cells, (Pappenheim), i.e., from Pappenheim's Grosslymphozyten, grosse Lymphozyten, Mesolymphozyten, kleine Lymphozyten and large leucocytoid lymphocytes (Lympholeucozyten, large mononuclears). The lymphocytes which develop into plasma cells may be of hematogenous or of histogenous origin, but the majority of them are of histogenous origin.

Plasma cells are also formed by direct differentiation of Marchand's perithelial cells, of Maximow's resting wandering cells (Marchand's perithelial cells or clasmatocytes) and small wandering cells of the connective tissue, of Saxer's primary wandering cells (Grosslymphozyten, Pappenheim), of large mononuclears which come from the mesothelial cells of the mesentery, and finally by direct differentiation of fibroblasts (Pappenheim).

The chief source of plasma cells is from the small lymphoid wandering cells which result from proliferation of the above types of cells.

In speaking of the development of plasma cells directly from fibroblasts, Downey says: "The plasma cells formed in this way are not numerous, and it is still a question as to whether they ever develop into the typical Marschalko type." Some recent workers are still divided in their views as to the origin and morphology of the plasma cells, although, as stated before, the generally accepted form or type is that described by Marschalkó. As late as 1919 Pappenheim (p. 188) described plasma cells as "histogenous granulation tissue lymphocytes changed by inflammation." Maximow ('22) calls attention to the development of plasma cells from connective tissue. The cells produced in this way do not, according to him, come from fibroblasts, but from lymphocytes which are developed from reticulum cells.

It is evident from the above brief sketch of the history of the plasma cell that there has been and is still a lack of agreement among workers as to its origin and morphology. It is not the purpose of this paper to seek to determine or to investigate all the possible sources from which plasma cells may arise or to go into a discussion as to the conditions giving rise to these cells or their function or purpose. It is sufficient, however, to show that plasma cells do originate directly from connective-tissue fibroblasts as claimed by Unna, that the cells produced in this manner are of considerable number, that these cells may attain the typical type or form as described by Marschalkó, and that, as Downey believes, the Marschalkó type of cell is not necessarily the only true plasma cell, but is only one expression of several different forms.

Observations

The nictitating membrane from any half-grown or adult cat shows great numbers of plasma cells and all degrees of their development from the surrounding and interlobular connective tissue (figs. 3 and 4). In some few cases plasma cells were seen developing from histogenous lymphocytes as shown in figure 6.

The first step observed in the development of a fibroblast destined to become a plasma cell, is the appearance of granuloplasm.[1] This granuloplasm is first seen at either or both poles of the nucleus. It does not appear thicker or denser at the margin or appear first at the margin of the cell, as claimed by some authors, who explain its development as a reaction of the cell to an external irritant. It has a homogeneous appearance, but in some cases a light perinuclear zone is present. With the increases in granuloplasm, the chromatin material of the nucleus becomes less scattered, the fine small particles grouping together in clumps which are arranged in no particular manner. With this clumping of chromatin, the nucleus tends to become less oblong and more spherical in shape. As these changes in the nucleus and the cytoplasm are taking place, the cell outline becomes less fusiform in shape and tends to take an oval, cuboidal, or round form, depending on its location; i.e., if pressed between other structures it is cuboidal or oblong, while if comparatively free it is more round or oval. With this change in shape of the cell, the nucleus has become round and the chromatin is, in some cases, arranged in such a manner that the typical 'Radkern' is formed. In other cases the chromatin material is not placed in this way, the clumps being more scattered and less definite in their arrangement. The nucleus is generally eccentric in position, but not always so, some cells being observed which had the nucleus in a central location. The perinuclear zone is, as a rule, very apparent. The granuloplasm takes a heavier stain as the cell becomes more 'mature,' but in all cases it is not the peripheral zone of granuloplasm which is the darker. The cells become cut off from the connective tissue and lie in clumps between masses of collaginous material or between gland acini and seem to be especially numerous in those areas in which degeneration is going on. The position of many suggests that they may be

[1] The application to the plasma cell of the term 'granuloplasm,' as used by Unna, Downey, and others, is done in this paper without any attempt being made to justify its use in this way.

ameboid, some of them being quite free from and clear of surrounding structures.

Those plasma cells developing from lymphocytes are very few in number in this region as compared to those developing from the connective tissue directly. These lymphocytes are first cut off from the connective tissue and are of the small type. They have a very narrow rim of cytoplasm, which, as it increases in amount, shows the presence of granuloplasm. The chromatin of most of the cells developing in this way becomes arranged in a 'Radkern' formation; the nucleus may or may not become eccentric, and a perinuclear zone is, in most cases, apparent (fig. 6).

The production of plasma cells is very slight, if it occurs at all, in the areas in which lymph nodules are present.

A few of the plasma cells show the presence of fuchsin bodies. Some of these cells are shown in figure 5. These bodies first appear as small droplets in the granuloplasm of the cell and not necessarily in any one region, although Downey found them always appearing in the lighter-staining area of the cytoplasm. In some cases I found these bodies located not in the lighter area, but rather grouped about it.

The droplets increase in size and decrease in number by coalescing. Some of them become nearly one-half as large in diameter as the nucleus of the cell itself, and, as the cell becomes filled with these bodies, it appears to disintegrate, the fuchsin bodies thus becoming free. Their formation suggests, as the work of Downey indicates, that they develop intracellularly, and not that they are ingested nuclear fragments, as claimed by some workers. It may be also suggested that the plasma cell is secretory in function and that these bodies "probably represent a special kind of secretion, or the accumulation and thickening of the normal secretion" (Downey). Many plasma cells were observed undergoing disintegration without the presence of fuchsin bodies.

It might be of interest to call attention at this time to the similarity in development between plasma cells originating directly from fibroblasts, and cells of Leydig or interstitial

cells of the Urodele testis, as observed by Humphrey ('21). Humphrey observed these cells develop directly from the stroma cells of the genital glands of these animals. The appearance of droplets or bodies in the cytoplasm having a strong affinity for acid fuchsin and osmic acid, the presence of a large centrosphere and an eccentric nucleus were also observed by him. He further noted that the presence of these cells bore a definite relationship to the degenerative changes occurring in these organs; i.e., their development and number to be in proportion to the degree of degeneration taking place. The great frequency of plasma cells in areas of degeneration, their connective-tissue origin, and their mode of development suggest that they may be the result of a reaction caused by a change in metabolism occurring in the particular area in which they are found. The presence also of the large centrosphere and the occurrence of fuchsin bodies lend support to the view held by some workers who believe them to be hypertrophied connective-tissue cells in an atrophic condition, and not a highly specialized cell of special secretion. While the evidence in favor of the former view is far from conclusive, it is, however, well deserving of consideration.

No indications of a reversion of plasma cells to the type of cells from which they developed were observed; i.e., I have seen no evidence that a plasma cell can, by a process of losing its granuloplasm or by any other process, become a fibroblast or a lymphocyte again and thus take up its former function, as claimed by some workers in this field.

Summary

A study of plasma cells in the nictating membrane of the cat results in the following conclusions:

1. Plasma cells are produced in large numbers directly from the fibroblast of the connective tissue of the membrane, and cells of this origin may attain the typical Marschalkó form.

2. Plasma cells are also developed from histogenous lymphocytes in this area, but the cells formed in this way are

very few in number as compared to those developing directly from fibroblasts. It is of course not improbable that, in other instances, the majority of plasma cells in a given tissue may pass through the lymphocyte stage in their development. In the material studied this did not, as related before, prove to be the case.

3. The Marschalkó type of plasma cell is not the only true plasma cell, but is only one of several forms.

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PLATE 1

EXPLANATION OF FIGURES

1 Showing area of cystic duct. The lower right half of the duct has its epithelium thickened and invaded by the lymphocytes, while the duct epithelium in the upper left area is unaffected. Zenker's fixation; 10 μ; hematoxylin and eosin. Cat. Photo $\times$ 288.

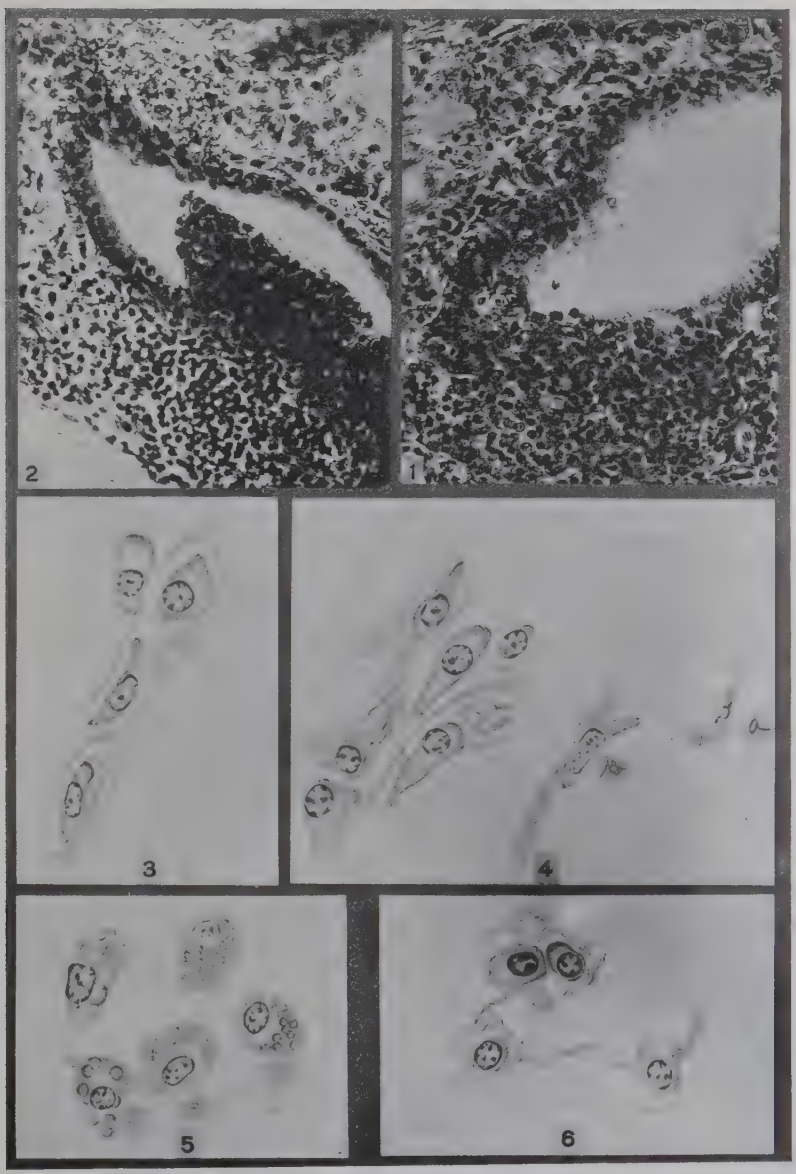
2 A portion of a duct. Note the epithelial thickening protruding into the lumen of the duct and invasion of the duct epithelium by the lymphocytes. The epithelium on the side of the duct free from lymphocytes is seen to be unaffected. Zenker's fixation; 10 μ; hematoxylin and eosin. Rabbit. Photo $\times$ 288.

3 A camera-lucida drawing of a field showing cells found in the interlobular connective tissue of the nictitating membrane of a twenty-year-old cat. The figure shows two fibroblasts developing into plasma cells and two others which have developed into the mature plasma cell of the Marschalko type. Helly's fixation; 5 μ; alcohol eosin and methylene blue. Photo $\times$ 1000.

4 Shows another area of the interlobular connective tissue of the nictitating membrane of a twenty-year-old cat in which the development of fibroblasts into plasma cells is apparent. Several mature plasma cells are present, although none are exactly of the Marschalkó type. The development of granuloplasm is just beginning in (a). (b) is a little later. Helly's fixation; 5 μ; Mallory's connective-tissue stain. Photo $\times$ 1000.

5 This figure shows the presence of 'fuchsin bodies' in different stages of development within the plasma cell. In the cells (a), (b), and (c) the bodies are not concentrated in the lightest area of granuloplasm as stated by some authors. Cat. Helly's fixation; 5 μ; Mallory's connective-tissue stain. Photo $\times$ 1000.

6 A field drawn from the same organ of the same animal used in figures 3, 4, and 5. The field shows stages in the development of histogenous lymphocytes into plasma cells. The upper two cells, especially the one on the right, are of the Marschalkó type. The lower two cells are histogenous lymphocytes in which the granuloplasm is just beginning to appear. Cat. Helly's fixation; 5 μ; Mallory's connective-tissue stain. Photo $\times$ 1000.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ON THE MUSCULAR ARCHITECTURE OF THE VENTRICLES OF THE ALLIGATOR HEART, WITH A NOTE ON THE FORMATION OF THE INTERVENTRICULAR SEPTUM OF BIRDS AND MAMMALS

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SIX FIGURES

In a previous paper the writer ('23) extended into lower vertebrates the work done on the mammalian heart by MacCallum ('00) and Mall ('11), and described the musculature of the ventricles in the fish, frog, turtle, and bird. The fowl heart was found to be in all essentials like that of pig and man. The rest form a series of increasing complexity. All three, of course, are furnished with a single ventricle. The chief muscle coat in the fish and frog is a circular one. The turtle ventricle, on the other hand, is provided with a coat of spirally running fibers, and a rudimentary muscular septum—two features not found in Ichthyopsida, but very highly developed in birds and mammals.

For such a study the alligator heart would have been very useful. It would have closed the gap between the turtle and bird, and would have helped one understand the formation of the interventricular septum. I have recently obtained through Mr. Percy Viosca, of New Orleans, adult hearts of the American alligator (A. mississippiens), and can now describe the architecture of its ventricles, and complete the analysis of the interventricular septum.

The general anatomy of the alligator heart is described in many text-books; the account of Owen ('68) is especially

useful. For the present purpose, the following points should be kept in mind. The apex of the heart is attached to the pericardial sac by a strong apical ligament. There are two ventricles, completely separated by a muscular septum. The internal wall of each ventricle is covered by irregular trabeculae, and that of the left bears small papillary muscles. The lateral valve of the right atrioventricular orifice is muscular—a peculiarity not found in mammals but exaggerated in birds. The right ventricle expels its blood into both a pulmonary artery and a left aorta. The left ventricle discharges into a right aorta. The two aortae are not of equal importance. The left one, from the right ventricle, has but one large branch, the coeliac artery. The right aorta, from the left ventricle, is the parent trunk for all other great arteries. The two aortae communicate above the semilunar valves through the foramen Piazzae. The foramen is above the valve, not below them, and does not pertain to the adult interventricular septum.

DISSECTION

The hearts had been preserved in a supersaturated solution of carbolic acid. Formalin should be avoided for this purpose. Before dissection they were soaked overnight in a very dilute acetic acid, and then allowed to simmer a half hour in the same solution. Boiling contracts the hearts too much. As the first step in dissection, the heavy pericardium was pulled off. The heart then appeared as in figure 1.

The surface of the ventricles is everywhere covered with a thin muscle layer, about a millimeter thick, the fibers of which arise around the great orifices of the heart and run directly toward the apex. Except over the dorsal side of the right ventricle this superficial muscle is loosely tied to the muscles beneath. It inserts into the apical ligament.

The superficial muscle of the alligator is the best example so far met within the vertebrate series. A thin muscle layer of the same sort is present in the sturgeon, and occasionally can be found well developed in the turtle. Isolated remnants

ABBREVIATIONS

A.B., sinospiral muscle
A.C., bulbospiral muscle
A.d.,s., right and left atria
A.o.d.,s., right and left aortic arches
A.p., pulmonary artery
A.v.s., left atrioventricular orifice
C.v.d., cavity of the right ventricle
L.a., apical ligament
L.v.d., longitudinal muscle of the right ventricle
O.b., bulbar orifice
P., large ventral trabecula
S., superficial muscle of the ventricle
V.a.v.d., right atrioventricular valve
X., deep bulbospiral muscle
Y., deep bulbospiral and sinospiral muscles

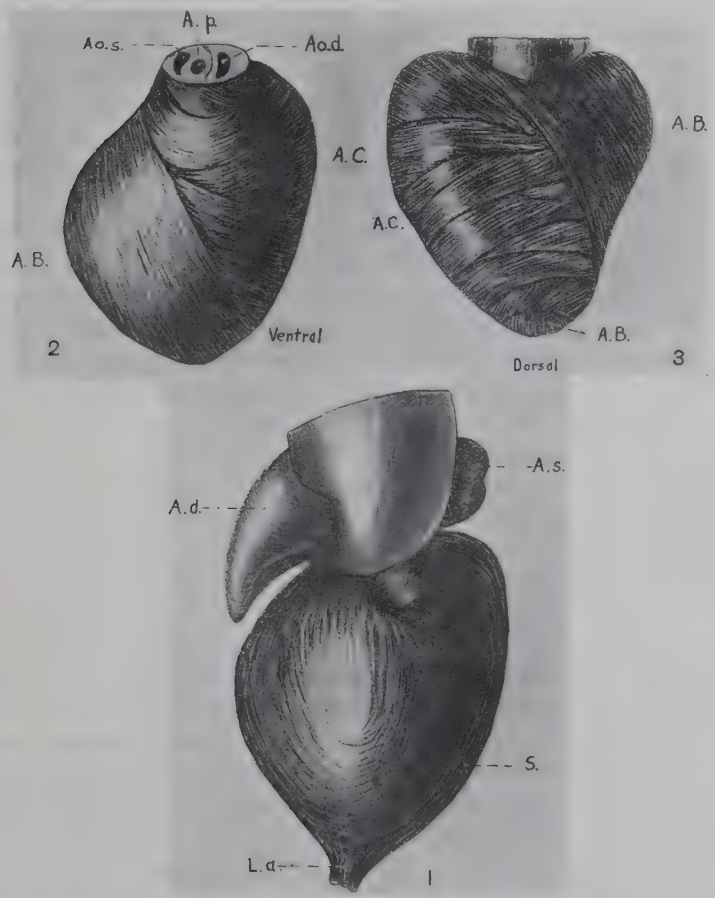

Fig. 1 Alligator heart. Pericardium removed to show superficial muscle layer of the ventricles. × 1.

Fig. 2 Alligator heart. Ventral view. Superficial muscle layer removed. × 1.

Fig. 3 Alligator heart. Dorsal view. Superficial muscle layer removed. × 1.

of it appear in some fowl hearts, and are found attached to the pericardium in man. The superficial muscle seems more closely connected to the pericardium than to the deeper muscles, and its great development in the alligator may be related to a tough pericardium and a stout apical ligament.

To continue the dissection: The atria and the great arteries were next cut off close to the ventricle. The superficial muscle layer was loosened around the heart base and pulled off. Except over the right ventricle where the superficial fibers parallel those beneath, the outermost layer comes off as a distinct sheet. The heart then appeared as in figures 2 and 3.

In general, the surface now exposed is formed of spirally running fibers, which appear around the great orifices of the heart and course in a sinistral spiral toward the apex. The individual fibers arise at the heart base; in part from definite tendinous origins as in mammals and birds, and in part as direct continuations of the internal muscle meshwork. The distribution of the fibers about the heart base is such that they can be divided into two groups. The first, or *sinospiral* group, arises from the dorsal margin of the atrioventricular ring (fig. 3), passes over the right shoulder of the heart base on to the ventral surface (fig. 2). Some of its fibers gain the dorsal surface a second time at the apex, and then turn abruptly into the heart interior. The second group of fibers, the *bulbospiral* group, arises on the ventral surface around the great arteries of the heart (fig. 2), passes over the left shoulder of the heart base, and spreads out over the dorsal surface. On reaching the dorsal surface, the bulbospiral fibers change from their apical spiral course to a more circular one (fig. 3), during which the fibers in small groups dip into the heart. The dipping in is especially marked along the dorsal interventricular groove.

A vortex, in the sense that obtains in mammals and birds, is not found in the alligator. As a whole, the spiral arrangement is of an intermediate character, more pronounced than in the turtle, and less so than in higher vertebrates. The

sinospiral muscle pursues a course much like the one it takes in birds and mammals, but the bulbospiral muscle does not mass at the apex in the same way. There is consequently no grand converging of fibers at this point, and no finished vortex.

To follow the fibers into the deeper parts of the heart, it was necessary to continue the dissection in the following manner. The three arterial trunks were separated from one another. A cut into the surface fibers was made along the dorsal interventricular groove. The cut was begun just above the apex, made deeper toward the base, extended into the right ventricular cavity, a little to the right of the interatrial septum. The right ventricle could then be peeled away from the left, until a muscle on the medial wall of the right ventricle stopped further progress. The heart then appeared as in figure 4.

The sinospiral fibers that disappeared at the apex in figure 3 can now be traced into the cavity of both ventricles, where they enter the muscular trabeculae. The majority of the fibers seem to pass to the left ventricle and to end in the papillary muscles.

The deep continuation of the bulbospiral fibers makes up a larger mass, subdivided in the figure into the parts labeled *A.C.*, *X*, and *Y*. The part *A.C.* is a thin sheet, composed of fibers that have dipped inward to the left of the cut. These fibers end at varying distances by entering the internal muscle network and papillary muscles of the left ventricle. The basal part of this area (*X* in fig. 4) is a stouter group, which extends around the ventral and left sides of the left atrioventricular orifice, taking a course much like the deep bulbospiral muscle of mammals and birds. The part marked *Y* is an artificially separated part of the two preceding ones. Its fibers come from both surface groups. Some superficial fibers on the right side enter the muscle network of the right ventricle, the majority, however, end with the parts marked *A.C.* and *X*.

The same dissection discloses two lesser subdivisions of the heart muscle. The longitudinal muscle of the right ventricle (*L.v.d.*) arises from the base of the pulmonary artery and right aorta. It loses itself on the ventral wall and adjacent floor of the right ventricle. From its origin the muscle can be classed as a part of the bulbospiral system. The muscle of the right atrioventricular valve (*V.a.v.d.*) is a subdivision of the sinospiral muscle. It forms the fleshy mass of the valve, and ends on the ventral heart surface.

In the two last-mentioned features, the alligator heart shows its intermediate position between the turtle and the bird. The longitudinal muscle of the right ventricle is homologous with the ventral trabecula that forms the partial septum in the turtle (fig. 5); on the other hand, it resembles closely a muscle of the same name in the bird (fig. 6) and in man (Mall, '11). The muscle of the right atrioventricular valve is a sauropsidan structure foreshadowed in the turtle and greatly exaggerated in birds (fig. 6), but not found in mammals.

SUMMARY

To sum up a rather detailed description, the heart of the alligator is made of the following muscles: 1) superficial muscle; 2) sinospiral group: sinospiral muscle, muscle of the right atrioventricular valve; 3) bulbospiral group: bulbospiral muscle, longitudinal muscle of the right ventricle.

The superficial muscle is a subpericardial layer superimposed on the heart muscle proper. Its fibers arise around the great orifices of the heart and run directly toward the apex. The muscle is inserted into the apical ligament.

The sinospiral muscle arises from the dorsal margin of the atrioventricular ring and passes in a sinistral spiral over the right shoulder of the ventricles on to the ventral heart surface. It reaches the dorsal surface a second time at the apex and then turns into the heart. Most of its fibers dive beneath the bulbospiral fibers to reach the papillary muscles and muscle network of the left ventricle. A few enter the muscle network of the right ventricle.

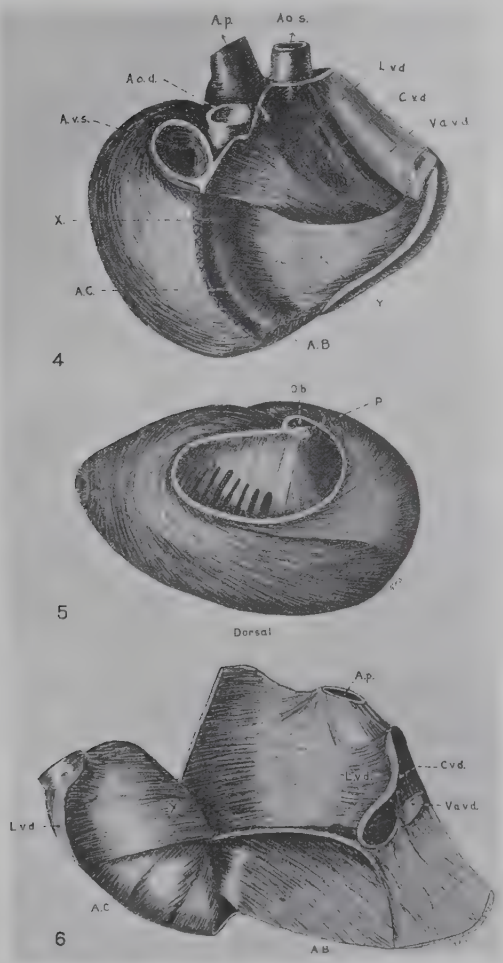

Fig. 4 Alligator heart. Dorsal view. Dissected to show deeper course of the sinospiral and bulbospiral muscles. × 1.

Fig. 5 Turtle heart. Dorsal view. From Shaner ('23). × 1⅓.

Fig. 6 Fowl heart. Dorsal view. From Shaner ('23). × 1.

The muscle of the right atrioventricular valve arises beneath the preceding and winds around the base of the right ventricle. It forms the fleshy mass of the right atrioventricular valve, and ends on the ventral surface of the heart.

The bulbospiral muscle arises around the great arteries on the ventral surface of the heart and then passes over the left shoulder of the ventricles on to their dorsal surface. Here the fibers take a more circular course, dipping in smaller groups into the deeper parts of the heart, especially along the dorsal interventricular groove. Within the heart the fibers form most of the interventricular septum. The apical two-thirds of the fibers enter the papillary muscles and muscular meshwork of the left ventricle. The basal third remains a definite bundle for a longer course, ending along the ventral margin of the right atrioventricular orifice. The latter portion corresponds to the deep bulbospiral muscle in birds and mammals.

The longitudinal muscle of the right ventricle arises at the base of the pulmonary artery and right aorta and ends on the ventral surface and floor of the right ventricular cavity. The longitudinal muscle covers the deeper parts of the bulbospiral muscle, and with it forms the interventricular septum.

THE INTERVENTRICULAR SEPTUM

A study of the hearts of the turtle, alligator, and fowl (figs. 4, 5, and 6), affords an explanation of the development of the interventricular septum in birds and, by inference, in mammals also.

The interventricular septum of the bird heart was shown in a previous paper (Shaner, '23) to consist of a longitudinal muscle of the right ventricle and a massive bulbospiral muscle. A figure from that study is reproduced in figure 6. It shows the two elements of the septum dissected apart.

In the first place, it may be asked, what is the purpose of the interventricular septum? The one duty always assigned to it is the separation of venous from arterial blood. If the discharge of such a passive function be its primary object, it

is strange that the septum is so intricately built of contractile muscle, and that in the highest reptiles, where the septum is fully formed, the separation of the two sorts of blood is only partly accomplished. After all, the heart is primarily a pump, and only secondarily a separator. We can better understand the development of the septum if we invert the conventional conception of it, and think of it as fundamentally a reenforcement to the heart muscle to help the ventricles drive out their contents; a device which only secondarily, with the rearrangement of the blood vessels, takes on the passive function of separating the venous from the arterial circulation.

The chief muscle coat of the ichthyopsidan heart is a circular one. The heart of the fish and frog contracts somewhat as the bulb of a camera shutter. The shock of systole is distributed over the entire ventricle.

In reptiles and in all higher vertebrates the circular coat is replaced by one made of spirally running fibers. The external musculature of the turtle (fig. 5) is formed of such spiral fibers that arise as continuations of the internal trabeculae on the same side of the heart and end in the trabeculae of the opposite side. The contraction of such a heart wrings out the blood. But it also directs the full shock of systole toward the base of the ventricle. There is danger, as Mall put it, that the shock of systole might "shoot the aorta out of the heart." Hence one finds in all reptiles above Sphenodon a specialized trabecula, that is rooted below in the ventricular wall like other trabeculae, but attached above to the base of the bulbar arteries. The heart of the turtle contains a good example (fig. 5). The special trabecula ties down the heart base, and at the same time increases the pumping power of the ventricle.

For its immediate purpose the exact position of the reenforcing band or trabecula is not important. In Ophidia and Lacerta (O'Donoghue, '18) the trabecula is rooted in the dorsal wall of the ventricle and attached between the two aortae on one side and the pulmonary artery on the other.

In Chelonia it is rooted on the ventral wall and attached between the pulmonary artery and the left aorta on one side and the right aorta on the other. But, once in place, the new trabecula becomes a 'septum,' which partly subdivides the ventricular cavity, and by its attachment to the bulbar arteries throws their orifices to one part of the cavity or the other. In Ophidia and Lacerta the right cavity empties into the two arotae and the left into the pulmonary artery. In Chelonia the right cavity empties into the pulmonary artery and the left aorta, and the left into the right aorta.

There is considerable evidence to support the hypothesis that the alligator and the bird heart were derived from the turtle type. The special ventral trabecula of the turtle is homologous in position and relations to the longitudinal muscle of the right ventricle in the two higher forms. In all three the right aorta alone arises from the left ventricular cavity and feeds the entire systemic circulation. The left aorta and pulmonary artery arise together from the right cavity. The left aorta in the two reptiles supplies only the intestines; in the bird it disappears in foetal life.

In the Crocodilia a new functional difficulty seems to have arisen. The left part of the ventricle has to overcome, through the right aorta, the resistance of the entire systemic circulation, while the right cavity has only that of the pulmonary and intestinal vessels. In the larger, more advanced, and on the whole more active tail-swimming Crocodilia, the load upon the left ventricular cavity must have been greatly increased. The increase in load seems to have been taken care of by a further development of the bulbospiral muscle. Its dorsal fibers extended into and across the interventricular opening, thus gaining a hold on more of the trabeculae around the left cavity, and attached themselves to the ventral rim of the left atrioventricular orifice. Thus the deeper parts of the bulbospiral muscle formed a new spiral muscle that can empty the left ventricle independently and with greater power.

The extension of the bulbospiral muscle across the interventricular opening brought the muscle in contact with the longitudinal muscle of the right ventricle. The two then formed an impervious interventricular septum.

In the bird (fig. 6) development continued in the same direction and for probably the same reason. The bulbospiral muscle increased in size, encroaching still more along the inner surface of the left cavity. The longitudinal muscle of the right ventricle was also strengthened. Instead of dissolving into a general network on the inner wall of the right ventricle as in the alligator, it was extended out on to the ventral surface of the left ventricle, and became a powerful band to aid in the wringing out of the left ventricle.

At the same time, the obliteration of the left aorta accomplished what the interventricular septum did not—the complete separation of the lesser and greater circulations.

The interventricular septum of the bird heart is composed of two distinct elements, the longitudinal muscle of the right ventricle and the deep continuation of the bulbospiral muscle. From comparative studies it is clear that the first is derived from the special ventral trabecula of the turtle sort of heart, while the second represents a later extension of the superficial bulbospiral fibers across the remaining interventricular opening—a feature first found in Crocodilia. Both elements seem to have appeared in response to mechanical needs. The first element strengthened the heart base when the spiral system of fibers came into use. The second represents a compensatory growth which accompanied the increase in load upon the left ventricle in Crocodilia.

The interventricular septum of mammals is composed of the same elements, although in slightly different proportions. The bulbospiral muscle is even more highly developed than in birds, while the longitudinal muscle of the right ventricle has remained in a simpler state more like that of the alligator. It is true the left aortic arch becomes the systemic arch in mammals, but the left arch takes origin from the left ventricle, not from the right as in birds, so the mechanical require-

ments of the mammalian heart remain those of the bird. What has been said of the bird heart, therefore, probably applies to mammals also.

To those interested in mammalian embryology the question naturally arises, how nearly do the embryological stages of the mammal heart follow these comparative steps? This cannot be answered until the technical difficulties that surround such a study are overcome. In an organ the embryology of which so faithfully repeats the gross features of the hearts of lower forms, it is not unreasonable to expect a similar repetition in the history of its muscle. The data here presented afford at least a good working hypothesis until the development of the muscles in the mammalian heart can be traced out.

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EXPERIMENTAL HISTOLOGICAL STUDIES

I. SOME FACTORS REGULATING THE MORPHOLOGY OF TISSUE (URETER IN VITRO)

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TEN FIGURES

Experimental biology has received in recent times a most important method in the tissue culture. In May, 1910, in Harrison's laboratory, Burrows used blood plasma for the first time as a medium for cultivating the tissues of frogs. He also cultivated for the first time the tissues of a warm-blooded animal (the tissues of chick embryos in plasma from the blood of adult chickens) and observed mitoses in cells. He thus established definitely a means of cultivating tissue.

The blood plasma has very special technical advantages over the lymph which had been used previously by Harrison in that it can be obtained in quantity and from any of a large number of animals. The proof of the great interest which this work aroused is that about 400 papers have appeared between May, 1910, and to-day. These 400 papers can be divided into three classes. The first class of papers contains a description of the method together with many modifications and a general description of the biological characteristics of the cells as they are seen in the peripheral zone of the fragments. The greatest number of the 400 publications are in this first class. In the second class, which contains a smaller

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number of these papers, the authors have made sections of these cultures and studied carefully all the morphological, histogenetic, and cytological changes which take place not only in the peripheral zone, but in the transplanted fragment. In the third class of papers the method has been used to attack bacteriological, serological, pathological, and pharmacological problems.

In spite of this very large amount of work which has been done, it is interesting that no one has come to any definite conclusions about the general characteristic reaction of cells in vitro and the relation of the behavior of these cells in the cultures to their behavior in the body. Both Chlopin ('22) and Rhoda Erdmann have noted this fact. Champy found epithelial cells after a few days to assume a spindle shape. In the same way he found muscle, bone, and cartilage cells transforming themselves into cells resembling simple fibroblasts. Champy thought these cells had dedifferentiated or, in other words, they had returned to a common primitive type. In the body he thought differentiation is the result of forces acting about these cells. The new medium releases the cells from these forces and returns them to one common type. While Uhlenhuth and Foot have accepted this theory, other authors do not believe that cells lose their specificity in the cultures (see papers by Congdon, Carrel, Lambert, Hanes, Oppel, Ebeling and Fisher). A third group of authors, Awrorow, Timofejewski, Chlopin, Maximow and Atterbury, find no dedifferentiation. They find that the cells may undergo a further differentiation in the cultures, the same as they do in the embryo.

The question arose, what is the cause of these differences of opinion and results; is it due to differences in the technic used or to other factors? After a careful study of sections of cultures prepared by Champy and others of my own in the laboratory of Tellyesniczky, I concluded that these differences in the behavior of the cells described by different authors are wholly the result of the technic which they used, and not differences in the interpretation of the same results.

To prove this more definitely, I have undertaken a study of the behavior of the cells of the ureter of young rats in various kinds of culture media.

MATERIALS AND METHODS

The ureter was chosen as a tissue very suitable for this study, because it contains epithelium, muscle, and connective tissue. These are the three tissues about which we find the greatest divergence of opinion in the literature. The ureters used in these cultures were taken from three-weeks-old white rats. At this age the rats measured 6 to 8 cm. in length. The middle centimeter of each ureter was used. This was cut into two pieces. One half was fixed and sectioned to determine its normal histology, the other half was cut into small pieces and tested in the culture media. These ureters are very small and dry quickly. In order to obviate this drying, I removed them from the animals and cut them in a dish of 0.73 per cent salt solution. The ureters were removed from the rats when they were living and under ether. Eight rats were used for these experiments, and in each case a part of both ureters was cultured. From each rat ten experimental cultures were prepared and two pieces were sectioned to determine the normal histology.

Four different media were tested in these experiments. In the first series of experiments chicken plasma was used, which I found already prepared in the laboratory in large quantities. The plasma which I used was eight months old. It had remained in small glass tubes in the ice-box during this time. These tubes were not paraffin lined. They were ordinary glass tubes which had been well cleaned with soap water, acid, and steam. In none of these tubes did one find that any clotting had taken place. The plasma, on the other hand, clotted quickly when the rat tissue was added to it. According to Burrows, paraffin is not necessary for the preservation of a fluid chicken plasma. This plasma had been prepared by him (Burrows, '19). I also found that it is as easy to prepare the chicken plasma in clean glass tubes

as in paraffin-lined ones and that the cells grow as well in the old as in fresh plasma. For all these studies I used hanging-drop cultures according to the original method of Burrows published in 1910. Burrows has found that a salt solution 0.73 per cent is physiological for rat tissue, while chicken tissue grows better in 0.9 per cent solution. For this reason, when I used chicken plasma, I diluted it with distilled water so that its osmotic pressure became equal to a salt solution of 0.73 per cent. This last per cent of salt solution he has also found to be the best for all mammalian tissues, including man.

In all experiments with plasma I diluted the plasma from one-quarter to one-half with the salt solution which is isotonic for rat tissue. This I did because Burrows ('13) had found that the zone of proliferation is increased by the addition of salt solution. The cause of this increase in the migration and growth is due in part to the action of the NaCl and in part to the dilution of the fibrin portion of the plasma. In the second series I used chicken plasma and an extract of rat embryo. The embryonic extract was made from a rat embryo 2½ cm. in length. The embryo was first cut into small pieces and ground in a porcelain mortar with salt solution. I obtained first a turbid fluid by this method. This was centrifuged and the clear supernatant part was used for the cultures. Where I used these extracts I added it one part to one part of plasma. In the third series I used rat plasma half diluted with salt solution. I found it very difficult to prepare pure rat plasma—it clots very quickly even in paraffin-lined tubes. For this reason, in the fourth series of experiments, I used plasma prepared from the blood to which a known quantity of sodium oxalate was added. In this last experiment I also added embryonic extract. To this embryonic extract I added sufficient calcium chloride to just precipitate the oxalate and reestablish the normal calcium balance of the total medium. In preparing such cultures I first placed one drop of oxalated plasma on the cover-glass; to this I then added one drop of the embryonic extract containing the calcium chloride. The two were mixed together

and the tissue added. The calcium oxalate crystals were left in the medium (Burrows, '11; Barta, '23).

In every experiment the fragment of tissue was transplanted from the first drop of medium to a second after twenty-four hours. Burrows has found that the clots will not liquefy about fragments of normal human adult connective tissue if they are transplanted after twenty to twenty-four hours from the first liquefied drop of medium to a second. After lying for this time at 37°C. in the first drop of plasma which they liquefy, they fail to liquefy the second, but grow in it. The same I found true for fragments of the ureter of rats, which also contain epithelial cells.

All cultures were studied in a warm box while living and afterward were fixed in Zenker-formol (Maximow's modification); some of the cultures were stained in toto with hematoxylin and others embedded in celloidin-paraffin and serially sectioned. These sections were stained with eosin-azure. In several instances I wanted to study the same culture stained in toto and later in sections. In such cases I stained the whole culture with hematoxylin and mounted it in cedar oil. After completing my studies of the whole culture, I then cut it loose from the cover-glass, embedded it in celloidin-paraffin, and serially sectioned it.

A STUDY OF THE CULTURES

The tissue used in all of these experiments consisted of fragments 1 mm. in length, cut through the whole wall of the ureter. Each contained epithelium, connective tissue, and muscle as they occur in the normal ureter of these rats, as shown in figure 1, the reproduction of a drawing of a longitudinal section of the ureter of a three-weeks-old rat. It is to be noticed that in these young rats the compact and refringent layer of protoplasm on the inner surface of the epithelium ('cuticula' of Hamburger) has not developed. It is also to be noticed that there are only two layers of muscle—an outer longitudinal layer and an inner circular layer. Otherwise the histology is the same as in the completely developed animals.

The reaction of the cells of the ureter in a medium of chicken plasma

In a medium of chicken plasma the connective-tissue cells migrate out from the fragment into the medium. These cells show two types of morphology. A part of them are elongated slender spindle cells set close together. Another part of them appear as irregular polygonal cells or as incomplete spindles or ovoid or round cells. Many of the smaller round

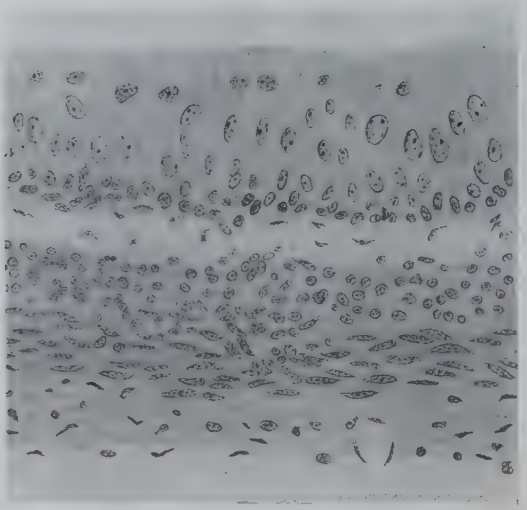

Fig. 1 Camera-lucida drawing of a longitudinal section of the ureter of a three-weeks-old rat, stained with hematoxylin, eosin, and orange G.

cells are evidently lymphocytes, while the larger ones are undoubtedly only connective-tissue cells. All of these cells contain fat-droplets and many of the more irregular polygonal cells also show phagocytosis of foreign matter. No evidence of growth or mitotic cell division was seen in any of the cells in chicken plasma. The long slender spindle cells are like the fibroblasts. A part of the polygonal cells resemble the so-called wandering phagocytic cells and another part are evidently the reticular cells of Maximow. A part

of these various types of cells are illustrated in the drawing (fig. 2).

Three rats were used for these cultures in the chicken plasma. Thirty cultures were prepared. The epithelial cells became active in only one of the thirty cultures. Serial sec-

Fig. 2 A camera-lucida drawing of the cells migrating from a fragment of ureter into chicken plasma. There are spindle-shaped fibroblasts, reticular cells of Maximow, and lymphocytes.

tions of many of these cultures showed that the epithelial cells in most cases had degenerated in situ. When the fragments are carefully cut, the epithelial cells are inside. In the one culture where they migrated out (fig. 3) the fragment had become turned inside out. In direct contact with the

chicken plasma, the epithelial cells had reacted. Away from it, they degenerate quickly, while the muscle and connective tissue remain intact for some time and show activity.

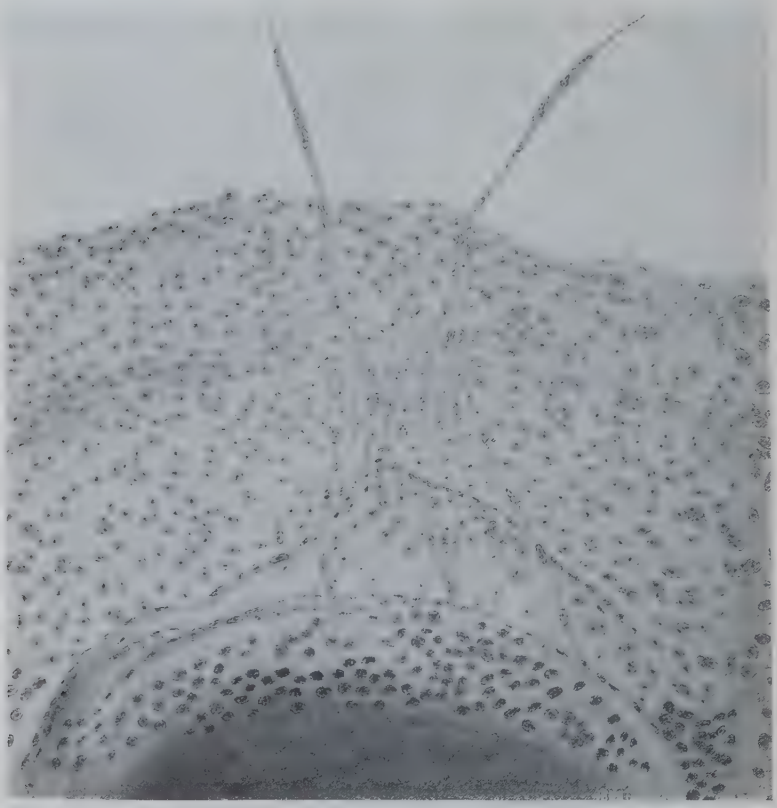

Fig. 3 A camera-lucida drawing of the one side of the fragment of the ureter in chicken plasma where the epithelial cells showed activity. A column of connective tissue is also migrating below the epithelial membrane.

In this culture it is also interesting to note that the epithelial cells did not liquefy the plasma and destroy the connective-tissue and muscle. It was possible, therefore, for the connective-tissue cells to migrate also from the fragments. This movement of epithelial cells and their failure

to liquefy the plasma did not occur in the first drop of plasma, but in a second. The fragment had been transplanted after twenty-four hours to the second drop. The picture was made on the fourth day of the sojourn of the fragment in the second drop of plasma.

The reaction of the ureter cells in a medium composed of one part of chicken plasma and one part of an extract of a rat embryo

Twenty cultures of this kind were prepared from the ureters of two rats. In this medium the epithelium instead of the connective tissue or muscle cells migrated out from the fragment into the medium. In about one-half of these cultures an epithelial membrane surrounded the fragment completely and in the other half of the cultures membranes extended out only from one side. In this medium the connective tissue failed to react in practically every culture. The balance between connective tissue and epithelial cells in this medium is the reverse of that seen in the pure chicken plasma. The epithelial cells are dominant in this medium, while the connective-tissue cells had dominated in the culture of pure chicken plasma (fig. 4).

In this latter figure it is to be noticed that all the epithelial cells have not remained in the membrane. A few have migrated into the medium to take a spindle or spherical shape. There was no evidence, however, that these cells had otherwise changed in character. They continued to stain like epithelial cells. As noted above, connective-tissue cells may also round off in parts of the culture. They likewise continue to stain like connective-tissue cells.

The reaction of the ureter in drops of pure rat plasma diluted one-half with 0.73 per cent NaCl solution

Only a few cultures were prepared in the pure rat plasma. I had considerable difficulty in keeping the rat plasma from coagulating. In these few cases the cells did not migrate

very actively. A section made across one of the cultures of this series is shown in figure 5. It is interesting to note that the tissues in this case retain their specificity almost completely. The epithelial cells do not degenerate. They migrate, but show no mitosis. The muscle and the connective tissue also retain their characteristic form. There was no evidence of dedifferentiation in this fragment. This section is made through the middle part of the culture.

Fig. 4 A camera-lucida drawing of the ureter in a medium of chicken plasma and embryo extract. A membrane of epithelial cells has spread out from the fragment. Isolated epithelial cells at the outer border of the membrane have taken spindle and spherical shapes.

The reaction of the tissue of the ureter in oxalated rat plasma and embryonic extract

In this series twenty cultures were prepared from the ureters of two rats. In these cultures as in the cultures of pure rat plasma the connective-tissue cells failed to migrate out in any number. One also sees a very meager outward spreading of the epithelial layer. Serial sections of these cultures are interesting. Within the fragment the epithelial cells have not degenerated. They have grown most actively and divided by mitoses (fig. 6). The muscle and the con-

nective tissue, on the other hand, has undergone degeneration of a hyaline-like type. One sees in many parts of this figure no nuclear material in the muscularis and the tunica propria. Note also in the drawing of the section how the epithelial layer has thickened irregularly and that the mitoses are located in the cells nearest to the hyaline connective

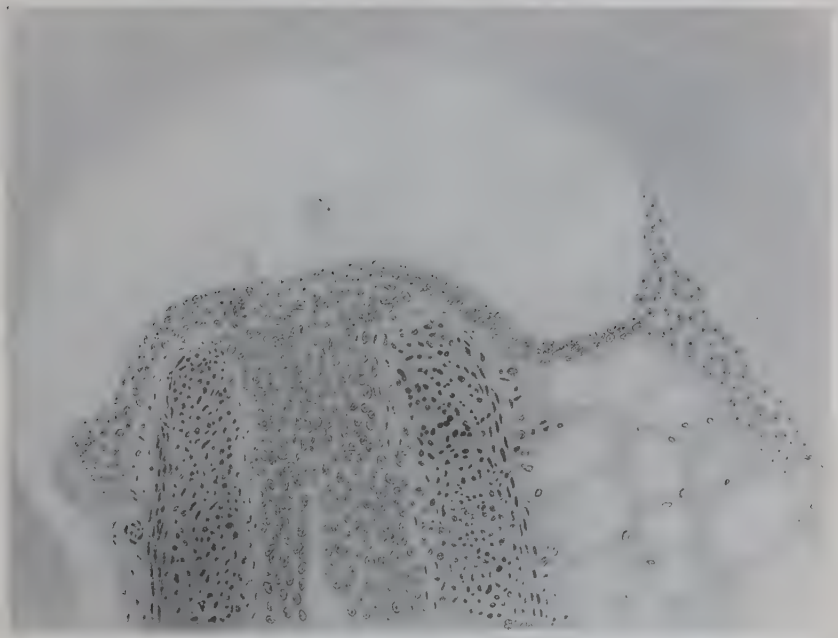

Fig. 5 A camera-lucida drawing of a section of a culture of ureter in rat plasma. All tissues have retained their specificity after four days in this medium.

tissue and muscle layers. In this culture from which the section drawn in figure 6 was taken the epithelial cells had remained in the central part of the fragment. There is also a considerable amount of retroperitoneal fat attached to one side of the fragment. In one section which passes through this fat I noticed that epithelial cells have migrated from the central region and have invaded this retroperitoneal fat tissue. Here they have grown and divided (fig. 7). In

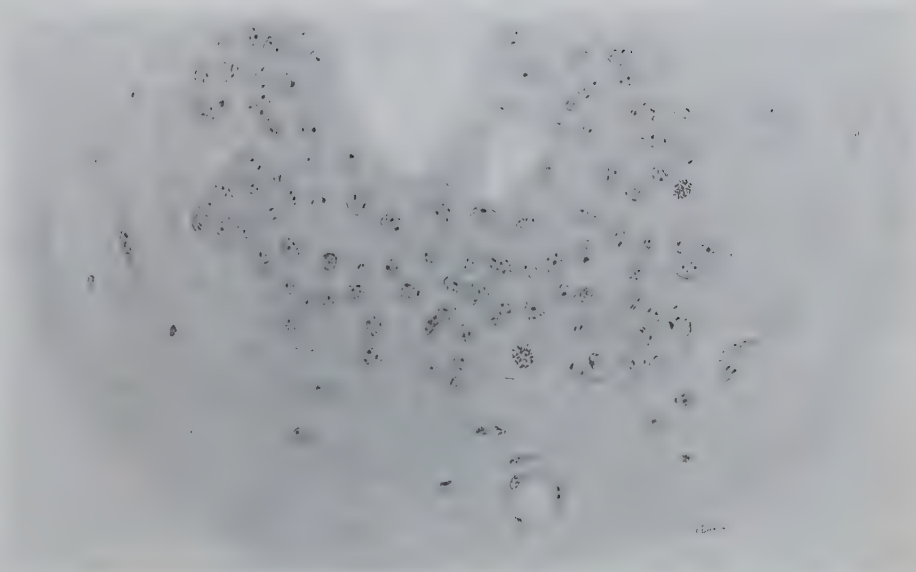

Fig. 6 A camera-lucida drawing of a section of a culture of ureter of the rat in rat plasma and embryo extract. The connective tissue and the muscle cells have degenerated. The epithelium is proliferating actively. Note the mitoses.

another section, which was cut through the periphery of this fragment (fig. 8), one sees that the epithelial cells have invaded a dense mass of connective tissue of the adventitia. Among these invading epithelial cells one finds mitosis and there are also multinuclear cells. The epithelial cells invading the connective tissue and fat have poorly defined margins. The invasion is quite typical of cancerous tissue. In other

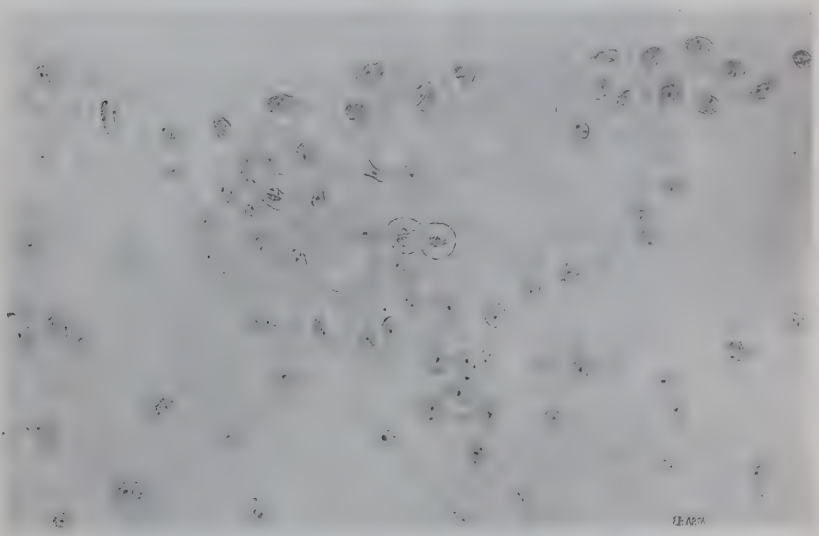

Fig. 7 A camera-lucida drawing of a section of ureter in rat plasma and embryo extract. The epithelial cells have spread from the center of the fragment of ureter and invaded the retroperitoneal fat-tissue of the fragment. Many mitotic figures are seen in these invading cells.

parts of this culture, where one finds no invasion of the fat by epithelial cells, other changes in the fat-tissue are interesting. In place of the fat-cells one finds an increased number of cells like fibroblasts and mitosis in these cells. The fat-cells have apparently lost their fat and returned to their more primitive type.

To complete the demonstration of the difference in morphology of the same tissue in different media, I have included

two microphotographs. In a medium of pure chicken plasma one sees that the connective tissue and the muscle of the ureter is most active and has proliferated. The epithelium is degenerated in this fragment (fig. 9). In a medium of rat

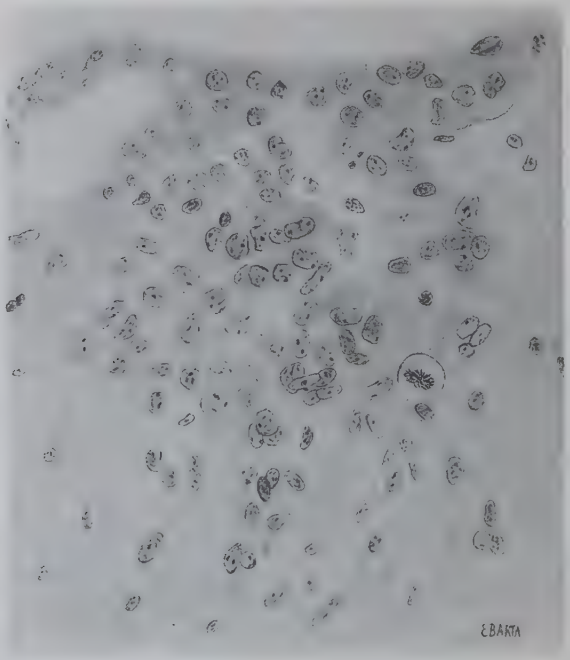

Fig. 8 A camera-lucida drawing of another section of a culture of ureter in rat plasma and embryo extract. In this section the epithelial cells have invaded the connective tissue of the adventitia of the fragment. There are mitosis and multinuclear cells as in cancer.

plasma containing embryonic extract (fig. 10), the epithelial cells are more active. In this section the epithelial cells have grown around the edge of the fragment and are invading the degenerated connective tissue and muscle of the ureter, *X*. At *Y* in this figure one sees one of the mitotic figures drawn in figure 6.

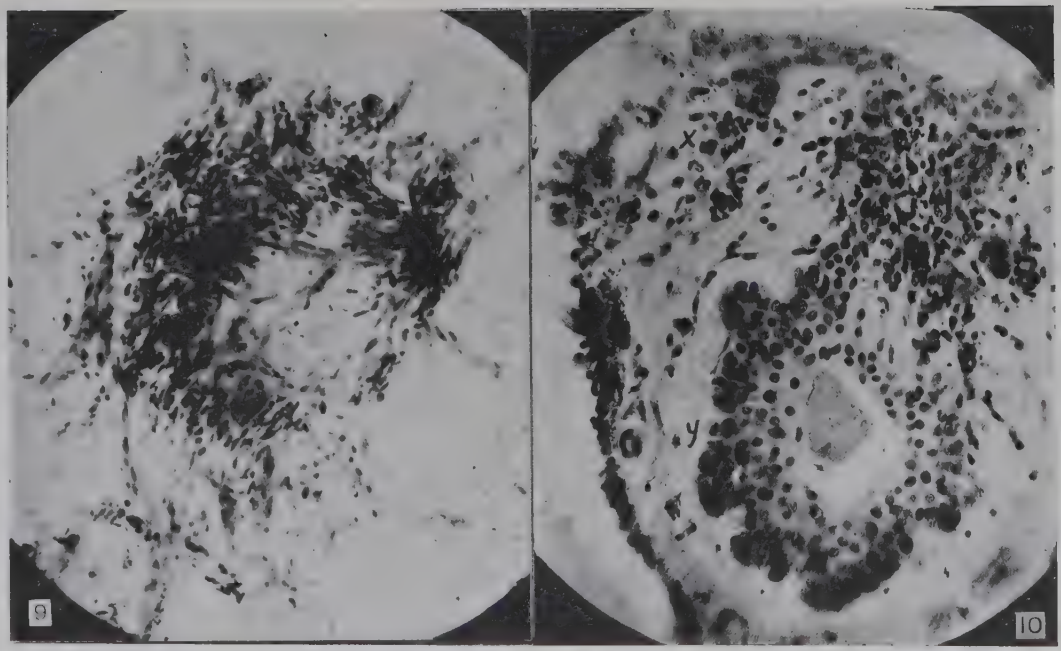

Fig. 9 A microphotograph of a section of the ureter in chicken plasma. It shows the connective tissue and muscle of the ureter suffering proliferation like the epithelial cells in the figure 10.

Fig. 10 A microphotograph of a section of the ureter in rat plasma and embryo extract. This section shows the proliferation of the epithelial cells. They are surrounding and invading the adventitia and one sees the degeneration of the connective tissue and muscle. Note the invasion of the adventitia by epithelial cells at *X*. A mitotic figure is shown at *Y*.

DISCUSSION

A careful survey of the picture of the various cultures studied in this series of experiments shows a variety of histological changes taking place about identical fragments of the same tissue of the same animal. Two variations which are associated with these changes in morphology are the medium and the arrangement of cells in the fragment. In a medium of chicken plasma the connective-tissue cells were alone active in fragments of the rat's ureter, excepting where the ureters were turned inside out and the epithelial cells were brought into contact with the medium. In the cultures where embryonic extracts were added to the medium there was a reverse dominance of the epithelial cells. In the cultures where the heteroplasma was used, the epithelial cells degenerated, the connective tissue remained. In the presence of the embryonic extracts the epithelial cells survived and destroyed the connective tissue and muscle. Burrows ('17) has shown that the cells in fragments of heart muscle which are in contact with the medium may live and grow at the expense of other cells within the fragments. In cultures of adult skin the epithelial cells frequently destroy the connective-tissue cells of the fragment in any medium. In cultures of embryonic skin where the mesenchyme is the more densely cellular mass the mesenchyme cells frequently destroy and live on the degenerating epithelial cells. In my experiments I have found the epithelial cell degenerating most frequently except where they are on the outside of the fragments or where the medium contains embryonic extracts. Where they are on the outside of the fragments, they migrate into the pure plasma. Where embryonic extracts are added to the medium, they prey on the connective tissue and muscle cells of the fragment.

There is no evidence in any instance that these epithelial cells revert to a connective-tissue type. They disappear simply in the presence of actively growing connective-tissue cells in certain media and under certain conditions. Under other conditions and in other media these cells either migrate

into the medium or proliferate in situ and invade the fragment. Where embryonic extracts are added to the media, these cells proliferate in situ at the expense of the connective-tissue cells. They also invade the connective-tissue areas exactly as cancer cells invade the tissue of a host. These invading cells proliferate actively and in many cases form large multinuclear cells. They have no basement membrane, but lie directly in contact with the connective-tissue cells which they are attacking.

During the growth of these epithelial cells the muscle, connective tissue, and endothelial cells are destroyed. In embryonic fragments Burrows had seen a similar active proliferation of mesenchyme cells and destruction of epithelium and blood vessels in a medium containing embryonic or tumor extracts. He has transplanted such fragments into the subcutaneous tissue of a host. When they are transplanted at the proper time they continue to grow as sarcomata and kill the animal. Fragments of these sarcomata also grow and kill other hosts as often as they are transplanted to them. There is little doubt, therefore, that we will find that these adult fragments, in which the epithelial cells are dominating and growing, will continue to grow as true cancers if they are transplanted at the proper time into a host.

The muscle and the connective-tissue cells which migrate into the medium lose all form peculiar to them in the ureter. This does not mean that they have changed their type. It simply means that these cells are simple fluid systems which become molded into shapes peculiar to their contacts. Epithelial cells coming into contact with the side of a fibrin fibril also assume a spindle shape on account of the shape of the fibril, and not because they have changed their type.

The changes which these cells suffer in the cultures are not to be called dedifferentiation in the general sense as it is used. These cells never change from one type into another. They suffer only a change in shape and in activity. We must assume that the differentiation of types or specialization is something peculiar to the embryo, but not to these types of

cultures. Whether these cells are to grow and divide or to function is, on the other hand, merely a question of adaptation to their immediate environment. Burrows has demonstrated this fact in a study of embryonic heart-muscle cells. By slight changes in the mechanical arrangement of cells he was able to transform a contracting heart-muscle cell into a growing cell which was not distinguishable from a sarcoma cell and vice versa. The contracting cell is simply a growing or migrating cell with its metabolic reactions localized at one end. It is a cell which has become polarized through specific peculiarities in the organization of its environment. Champy thought that the spindle-shaped epithelial cells which I have pictured in figure 5 had changed to a connective-tissue type. There is no evidence whatsoever that this is true. They are still epithelial cells. They take the characteristic stain. They have simply changed their shape as they are brought into contact with surfaces different from those of the body. In the same way I can find no evidence that the muscle cells do more than change their shape in the culture. The actual differentiation which determines the different layers in the embryo is not disturbed in these cultures. Again, this change in shape of the epithelial cells takes place only in certain types of media. It is not a general type of change as Champy thought it to be. The words 'dedifferentiation' and 'greater differentiation' as they have been used by different authors are misleading. I think it better to call these changes merely 'adaptation to the medium.'

In further proof for these deductions, Burrows ('13–'19) has shown that it is possible to reproduce readily the same reaction about any group of fragments of the same tissue having the same cellular content. He accomplished this only, however, when he was careful to cut the fragments the same size and to place them in the same thickness of layer of the same medium.

The tissue culture is nothing more than a biological reaction between two distinct phases: 1) the tissue fragment or cells and, 2) the medium. The comparisons become possible only

when both factors are understood. The morphological characters peculiar to no. 1 can always be modified by no. 2, the medium. In the first factor we must consider the animals from which the tissue is taken, the age of the animal (embryo or adult). In the second factor we must know the behavior of the cells to the various constituents of the medium. If we are going to criticise the various results of the different authors, we must use their technic. A proof of these deductions is the story of the cultures of the kidney in the literature. Champy found dedifferentiation when fragments of adult kidney are planted in pure plasma. Before Champy had begun his work, Burrows and Carrel had noticed tubules growing into the plasma from one out of a large number of cultures of the thyroid gland. In all other cultures the epithelial cells spread out as a membrane of cells. Atterbury found recently that when he plants pieces of nephrogenetic tissue of very young embryos on the area vasculosa he obtains a differentiation which looks much like that of the kidney. We can see from these experiments the various effects of the medium or the environment on the morphology of the cells.

By my experiments I have shown three distinct types of adaptation of the epithelial cells. The first type is shown in figure 4. In this figure one sees the epithelial cells spreading out as a membrane from the fragment and migrating out as single spindle-shaped and spherical cells. Figure 6 illustrates another form of adaptation. It is a drawing of a section of a fragment from a culture in which no epithelial membrane spread out from the fragment into the medium. The cells in this fragment have grown in situ. This growth is located chiefly in the cells nearest to the connective tissue and muscle. Corresponding with this activity in the epithelial layer, the connective tissue and muscle have undergone degeneration. A third type of adaptation of the epithelial cells to the cultures is illustrated in figures 7 and 8. In these figures one sees the migration and the growth of the epithelial cells into the connective tissue and the fatty tissue of the fragment. This type of reaction is typical of cancer.

In a few of the cultures where embryonic extract was added I have also noticed interesting changes in the fat-tissue. One sees fibroblasts forming among the previously fat-laden cells. One also sees in a few places in sections of similar cultures areas in the periphery of the fragments where the connective-tissue cells are proliferating. This proliferation is never so active, however, as that seen in the epithelium. The epithelial cells dominate in the cultures of the ureter when embryonic extracts are added to the medium. Only when the connective-tissue cells are widely separated from the epithelial cells do they become active.

From these studies it is evident that the type of reaction seen in any cell may be varied by changing the medium as well as the arrangement of cells in the fragment. It was this fact that led me to change the words and phrases 'greater differentiation,' 'dedifferentiation,' 'anaplasia to connective tissue,' 'returning to the embryonic type,' etc., to the expression 'adaptation to the media.' Using this phrase, we can eliminate the discussion in the literature and the great differences in opinion in regard to the morphological changes occurring in the tissue cultures and conclude that the different results obtained by different authors is only what is to be expected from the media and tissue they used.

Maximow has stated in a recent publication ('24) that he has seen breast epithelium grow and invade the connective tissue in a fragment of breast planted in a medium of blood plasma and an extract of bone-marrow. He could not reproduce this picture in fragments of the breast planted in a medium containing embryonic extracts. I have obtained the same result in the ureter planted in a medium of plasma and embryonic extract. Burrows has reproduced it with extracts of cancerous tissue.

In recent experiments Burrows has shown that the active principle in the embryonic extract is a product of the oxidative reaction in cells. This product acts according to its concentration to induce migration and growth in cells. It accumulates in any tissues where the cells are crowded

together and their blood supply reduced. The energy of a tissue is proportional to the concentration of this substance. Cancerous tissue like embryonic tissue grows because the cells are crowded together in an area having a reduced circulation. This substance Burrows has called the 'archusia.'

CONCLUSIONS

1. I have studied the effect of the medium on the morphology of the cells. In different media the morphology of the same tissue and cells may be different.

2. About fragments of tissue which contain epithelial cells, connective tissue and smooth muscle (ureter) planted in a heteroplasma without embryonic-tissue extract the most active cells are the connective tissue and muscle cells. The epithelium degenerates in a few days. The effect of the embryonic extract in both heteroplasma and homoplasma has been studied. In heteroplasma one sees membrane formation by the epithelial cells, but no growth and division. In homoplasma one sees an active irregular growth and mitotic figures and an invasion by the epithelial cells of the surrounding tissue.

3. Proliferation of the epithelial cells in vitro as noted in the last medium is similar to the proliferation of carcinomatous cells in the body (figs. 7, 8, and 10).

4. With a different medium and the same tissue one can reproduce differentiation, dedifferentiation, and retain the original specificity. I have called these changes therefore merely 'adaptations to the media.'

5. Many of the various changes described as peculiar for the tissue cultures have been explained by the differences in the medium used. Rhoda Erdmann states that we must have a normal type for the culture. It must be stated that every author has found evidently a normal type for the medium and the tissue which they used. The picture that they have described is the normal one for their own particular cultures. In criticising the morphological results described by these different authors, it is necessary to take into account

the media, the tissue (embryonic or adult), the thickness of the layer of media, the age of the culture, and the extract which they used.

7. Within certain limits it has been possible to modify the morphology of tissues by using the different media which have been devised. These changes are merely changes in shape and physical structure, and not fundamental changes in the chemical characteristics of the cells.

In conclusion I am indebted to the Rockefeller Foundation for the opportunity given me to work in the United States, especially Dr. Clifford W. Wells, and I wish to express my appreciation to Dr. Montrose T. Burrows for the courtesy of his laboratory, his personal interest, and the instruction he has given me.

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CYTOLOGICAL STUDIES OF THE ISLETS OF LANGERHANS IN A TELEOST, NEOMAENIS GRISEUS

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SIX FIGURES

As a result of the demonstration of the presence of insulin in mammalian pancreas and its production for clinical use, renewed interest has been taken in certain glands of the bony fishes which Diamare and Laguesse and later Rennie considered to be homologous with the islets of Langerhans. By the use of the modern cytological methods developed by R. R. Bensley, Lane, and others, Slater Jackson has demonstrated that these glands which may be called principal islets, a name suggested for the larger of them by Rennie, contain cells having the same characteristics as the cells of the mammalian islets, and Macleod has shown that very large yields of insulin can readily be prepared from them, although these are insignificant or absent from the pancreatic tissue itself. McCormick and Noble have further investigated this source of insulin, and the first-mentioned worker has also described the exact position of the islets in a large series of fishes. In connection with this work islets removed from each type of fish were preserved, after fixation, in 80 per cent alcohol, for microscopic examination, and the findings will be reported later. The present report is confined to a description of the various islet cells in one species of Teleost, the gray snapper (Neomaenis griseus), the tissues from which were fixed by Zenker's solution modified as recommended by Martin, and stained by a neutral stain which is a modification

of Bensley's neutral gentian, in which basic ethyl violet is substituted for gentian violet and Biebrich scarlet for orange G.

In a review of the earlier researches on islet tissue in Teleostei, the work of Diamare, Laguesse, and Rennie stands out prominently. By applying modern methods of fixing and staining, our investigations have confirmed the identity of the structures described by them, and have also led to certain cytological findings, hitherto unrecorded in fishes. It may be noted here that the gray snapper (Neomaenis griseus) belongs to a group of Teleostei that shows greatest divergence from Elasmobranchii. It is very common along the Florida coast and has often been called Lutianus griseus, from which, however, it differs slightly.

TECHNIQUE

All tissue intended for microscopic examination was placed in the fixative as quickly as possible after death. This is important, since Carter ('17) has noted postmortem changes in the finely granular cells of developing teeth of the pike, unless fixed within three minutes after death.

Fixative. Zenker's fluid (modified).

Mercuric chloride	5	grams
Potassium bichromate	2.5	grams
Glacial acetic acid	2	cc.
Distilled water to	100	cc.

We found it most convenient to mix the dry salts in proper proportion in a mortar and then grind them to a fine powder. The powder was put up in weighed packages, sufficient to make 50 cc. of fixing fluid. The acetic acid and water were added to the powder, with shaking, shortly before the tissue was immersed, thus giving a perfectly fresh fixing fluid for each portion of tissue taken. With fixatives less fresh, differentiation of islet cells was not so good, and we have concluded that perfectly fresh solutions are an important consideration in islet-cell fixation.

Small pieces of tissue containing the pricipal islets were immersed in the above solution for from twelve to twenty-

four hours, washed in running water or many changes for twenty-four hours, and then passed through graded alcohols to absolute alcohol. To remove excess of mercuric chloride, a little iodine was added to the 70 per cent alcohol in the graded series. If this failed to remove all of the excess of mercuric chloride, sections fastened to the slide were treated with 0.2 per cent iodine in 80 per cent alcohol for two or three minutes and then with a 0.25 per cent solution of $Na_2S_2O_3$ in 50 per cent alcohol for a few minutes, as recommended by S. G. Scott ('12). After absolute alcohol, the tissue was placed in a mixture of equal parts of oil of cedarwood and absolute alcohol for three or four hours, and then embedded in hard paraffin.

Stain. Neutral ethyl violet, Biebrich scarlet.

Biebrich scarlet is an 'acid' dye, soluble in water, and combines with basic ethyl violet in the proportion of 1 to 2. Aqueous solutions were made of each dye and the one added to the other in the above proportions. The aqueous solutions were strained before combining, to insure against having undissolved particles of either dye in the mixture. The resulting precipitate—the neutral dye—was filtered and washed well with distilled water[1] after which it was dried in a current of warm air and then weighed. A stock solution was made up by dissolving a known weight of the dye in a measured quantity of absolute alcohol and, for staining, 1 mgm. of the neutral dye added to 100 cc. of 20 per cent alcohol was applied to the sections for twenty-four hours.

More complete details for making up neutral dyes have been given by Martin ('15).[2]

The paraffin sections were passed through xylol and graded alcohols to water and then stained for twenty-four hours They were then rinsed in acetone on the slide, to remove excess of dye, and differentiated by using Bensley's mixture

[1] Apparently the neutral dye is slightly soluble in water, as the filtrate continued to have a weak violet color after frequent washings.

[2] The ethyl violet was secured from the National Aniline Company, New York, and we were assured that it was identical with that used by Martin ('15). According to Martin, this dye is the hydrochloride of hexaethylpararosaniline,

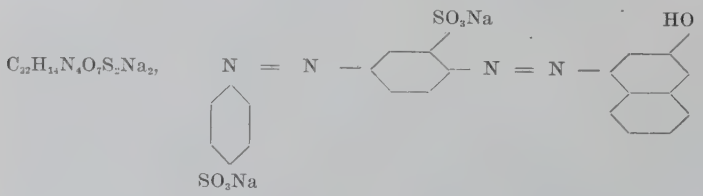
of one part of absolute alcohol and three parts of oil of cloves. Differentiation can be controlled under the microscope and may be prolonged without harmful results. Acetone may also be used, but being highly volatile, it is less convenient to handle.

Serial sections 4 μ thick were made for routine examination, but for more detailed study of granules sections of 2 μ were used.

DESCRIPTIONS OF SECTIONS

In common with many other types of Teleostei, the gray snapper has a number of spherical or ovoid nodules, made up of islet tissue, that are easily seen with the naked eye. The microscope reveals other smaller islets having the typical even contour, and also islets with only a few cells. The capsule, which is quite distinct in the larger islets, is absent where there are only a few islet cells, and these latter are often seen intimately surrounded by a band of zymogenous tissue (fig. 4). It is not unusual to find the large islets

Schultze no. 518. The Biebrich scarlet was from the American Aniline Products, Inc., New York. According to the manufacturer's description, this dye is the sodium salt of p-sulphobenzene-azo-O-sulphobenzene-azo-B-naphthol, having the formula



It is placed upon the market by the manufacturers under their trade name of scarlet red (medicinal). The dye is soluble in water, in striking contrast to scarlet red that is used as a stain for fat. The manufacturers state that "it receives a certain purification treatment" to add to its medicinal value. A sample of Biebrich scarlet from a different source proved to be less satisfactory.

After a great deal of correspondence and searching of records, it was found that there is much confusion in the available descriptions of scarlet red and Biebrich scarlet. The Commission on Standardization of Biological Stains, of Geneva, New York, recommend dropping the term scarlet red and using sudan IV (Schultz no. 232) for the fat-soluble dye, and Biebrich scarlet (Schultz no. 247) for the water-soluble dye, which is really the disulphonate of sudan III.

invaded by a projection of acinar tissue, just as the liver is invaded in these fishes.

It was soon found that the various types of islet cells could be identified not only by their staining reactions, but also in a general way by their cytological characters and by the position of the cells within the islet. In fishes the zymogen granules, and also those of the islet cells, are larger than the corresponding granules in mammals—a feature which aids in the histological differentiation of these types of cells. As a rule, medium-sized islets are most favorable for study, and for most purposes there is no advantage in picking out the largest. In many cases, where no supporting trabeculae invade the islet, a darker central area of irregular outline is seen surrounded by a lighter peripheral area, which in turn is usually bounded by a limiting capsule of even contour and frequently having narrow strands of zymogenous tissue attached to its outer surface. The special methods of technique for islet cells show that the central area is composed largely of densely granular alpha cells of Lane, while the peripheral area is made up of the somewhat less granular beta cells of Lane, and also decidely less granular and clearer cells that we have designated gamma cells. Whether the latter correspond to the gamma cells found by Bensley ('11) in the islets of the guinea-pig, we are unable at present to say.

The zymogen cells are, for the most part, arranged in elongated cord-like acini, with the zymogen granules occupying the inner zone of the cell and staining an intense blue color so that they stand out with shot-like distinctness in the grayish cytoplasm. The nucleus shows well, and the large single nucleolus in its center is very conspicuous.

Alpha cells. The alpha cells are usually found occupying the central areas of the islets. In small or medium-sized islets, which are often free from trabeculae, the central position of the alpha cells is more marked than in the larger islets (fig. 1). The relative number of alpha cells in different islets varies greatly. The smallest islets often do not show any alpha cells, whereas the medium or larger islets may show

about 50 per cent of them (fig. 6). Rarely is a small islet found that is composed chiefly of alpha cells.

With the technique described above, the alpha cells take the blue (basic) fraction of the neutral dye (table 1), the shade of blue being brighter and distinctly different from that taken up by the zymogen granules. That the blue-staining cells are those designated as alpha by Lane, Bensley, and Martin was shown, among other evidence, by the following:

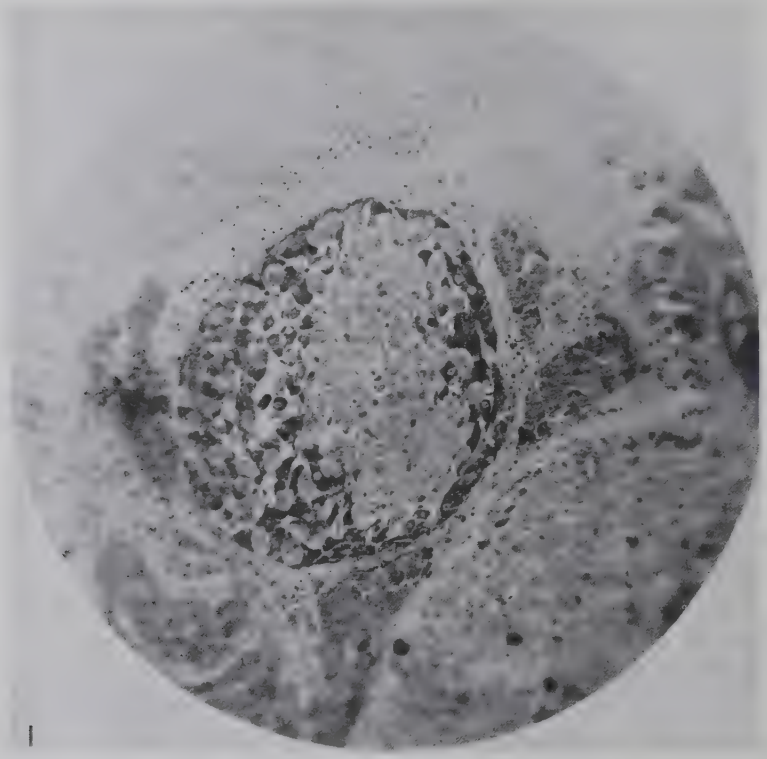


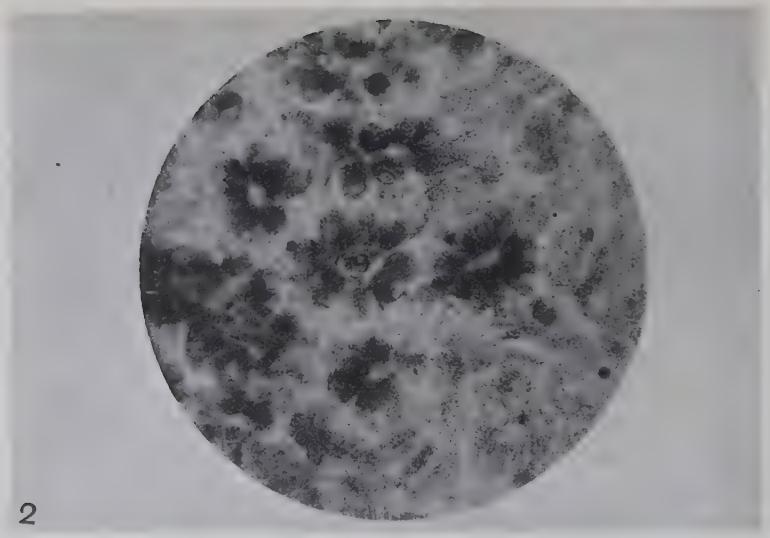
Fig. 1 This photograph shows a central area of alpha cells surrounded by an area of beta and gamma cells. The technique employed favors the beta cells which can be distinguished by the presence of dark granules. The gamma cells are intermediate in stain, the alpha cells lightest. Note the irregular outline of the beta cells and also the distinct central nucleolus. Several nucleated red blood cells may be seen in the area occupied by alpha cells. Zenker fixation (modified). Eosin-methylene blue. × 340.

1. This type of cell took up the blue (basic) stain, while the other types of islet cells did not do so in sections that were stained in neutral gentian after fixation in alcohol chrome sublimate solutions—the alpha cell fixative of Lane (fig. 6).

2. The cells which stained with the blue (basic) fraction of the neutral dyes as used by Martin ('22), after the modified Zenker fixation described above, give the same reaction as those found by him to correspond to the alpha cells of Lane.

The alpha cells are the most densely granular of the islet cells, and very thin sections are required to distinguish the granules clearly. The granules appear smaller and more uniform in size than are those of the other types of cells. The cells are fairly regular in outline, though the limits are not distinct, and they are often pear shaped, with the smaller end touching a capillary. When a capillary surrounded by alpha cells is cut in transverse section, the cells are seen to be radially arranged about the blood vessel much like the petals of a flower around the pistil (fig. 2). This rosette formation is best seen in medium-sized islets, and might be compared to the acinar arrangement of zymogenous cells, so that without special staining misleading interpretations might be made.

The nucleus, which is irregular in outline, is usually placed at the base of the cell, away from the capillary. Contact with a capillary is an almost constant feature of these cells, and the side of the nucleus on which the granular cytoplasm lies indicates the direction of the corresponding blood vessel. The capillaries seem to be of larger caliber where they are surrounded by alpha cells. Other islet cells, such as the beta and gamma of figure 5, may also lie along the capillaries, but there is not the same constancy of this relationship as in the case of the alpha cells. Along some capillaries cut in longitudinal section, one side of the vessel may be seen to be bordered by alpha cells, while the opposite side is bounded by other types of cells. If such a column is cut in transverse section, it is seen that one or more of the cells that make up the rosette are not alpha cells. In large islets the columnar



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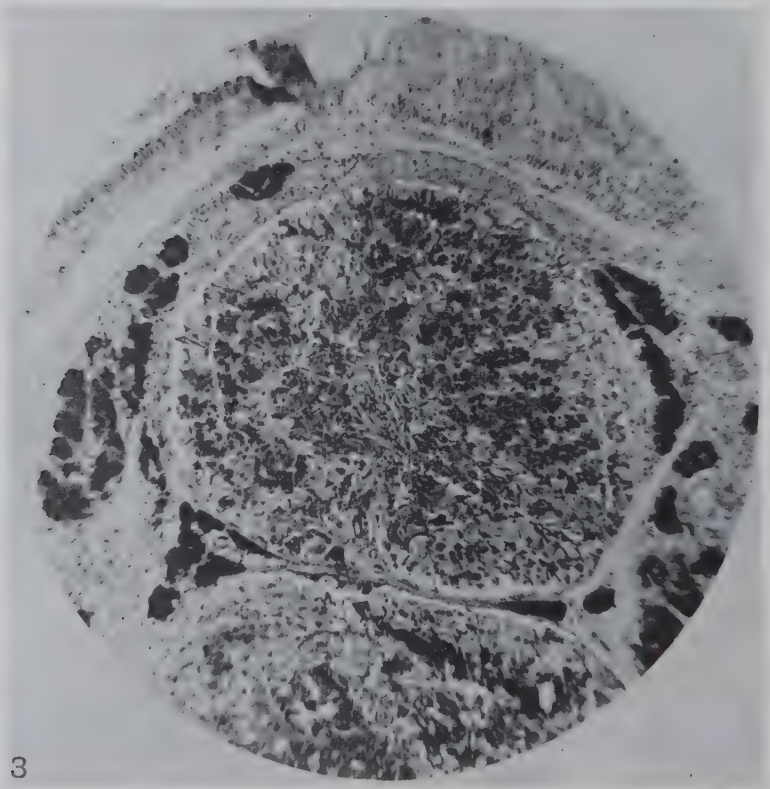

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or rosette appearance is not so marked, except toward the outer borders of the areas in which alpha cells are predominant. In the central parts of these, the alpha cells stand out more or less as individual cells, intermixed with other types, and their relation to the capillary is not quite so apparent.

While most of the alpha cells are crowded with granules, all gradations down to cells containing only a few blue granules may be seen. This variation of granule content has been described by Bensley in the case of the alpha cells of the guinea-pig. In very thin sections, indistinctly outlined, light-staining patches within the densely granular cytoplasm may be seen with oil-immersion lenses.

The nucleus, as stated above, is usually situated at the base of the cell, away from the capillary (fig. 2). It is often irregular in outline, being roughly elliptical and frequently kidney shaped, with the concave side toward the blood vessel. The nuclear membrane is quite distinctly seen in most of the cells. Neutral stains do not bring out chromatin material very distinctly, but alum hematoxylin shows the nucleus to be comparatively rich in that substance. There may be one or two small nucleoli, eccentrically placed. Occasionally there is seen a nucleus that is rather opaque and seems to take up the acid fraction of the dye.

Beta cells. After the Biebrich scarlet stain, the beta cells take a beautiful light purple color, with good contrast between

Fig. 2 This photograph shows the typical rosette arrangement of the alpha cells about a capillary. The alpha cell granules stain dark with this technique, and lie in the pole nearest the capillary; the faintly stained nuclei at the opposite or basal pole. Some of the vessels contain nucleated red blood cells. Zenker fixation (modified). Neutral ethyl violet Biebrich scarlet. × 600.

Fig. 3 This photograph shows a trabecula invading the islet from its lower border. Immediately to the left of the center is a somewhat triangular area of polygonal dark-staining beta cells. The darker-staining alpha cells, farther to the left, are separated from the beta cells by a narrow irregular band of light-staining gamma cells. The gamma cells also intervene between the alpha cells and the limiting capsule. The upper right portion of the islet is composed largely of alpha and gamma cells, while the lower right portion is made up of beta and gamma cells. The dark-staining areas outside the islet are acinar cells. Zenker fixation (modified). Iron hematoxylin. × 80.

them and the other types. For the most part, they are found toward the periphery of the islet and often may be seen closely attached to the limiting capsule (fig. 5). Where a trabecula invades the islet, the beta cells may be seen adjacent to and surrounding it, in which case they extend to the central parts of the structure (fig. 3). The beta cells are pyramidal or polygonal in outline, and are often seen to have one or

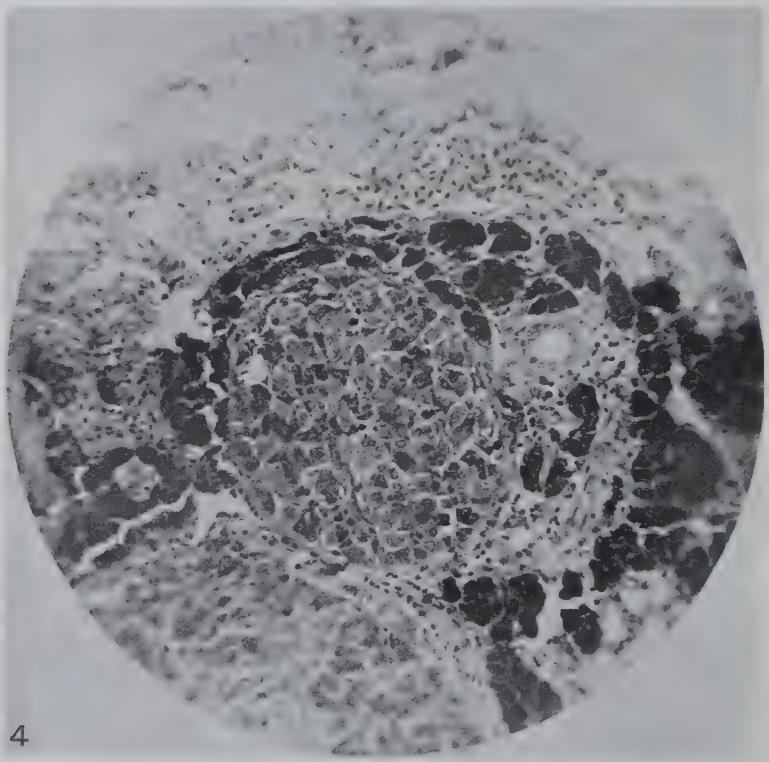
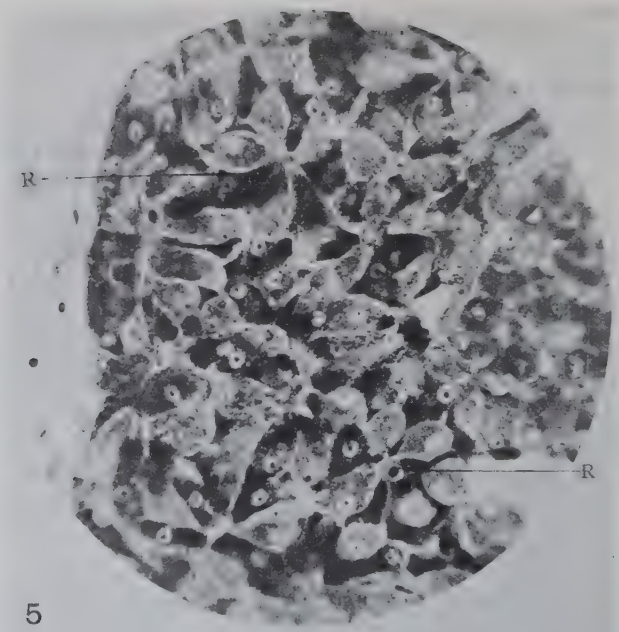


Fig. 4 This photograph shows a small islet composed largely of beta and gamma cells fairly evenly intermixed. In the lower right-hand part of the islet a few dark-staining alpha cells appear, two of which may be distinguished by their oval outline, touching a capillary containing a nucleated red blood cell. A duct appears to the right, outside the islet, while on the left are shown three or four islet cells surrounded by acinar tissue. Zenker fixation (modified). Eosin-methylene blue. × 340.

more slender, granular processes which insert themselves between gamma cells so as to touch a capillary (fig. 5). These slender processes frequently extend a considerable distance from the nucleus, and unless care is taken a cross-section of one of them may be mistaken for part of another type of cell. This indicates the necessity of having specific stains to identify the various granules. In certain islets the beta cells may be grouped in small, compact areas (fig. 3), and in such cases the cell outlines are polygonal rather than pyramidal. The medium and smaller islets often show the beta and gamma cells about evenly intermixed (figs. 4 and 5). One may also find beta cells lying in rows along a capillary, but this arrangement is not typical. The proportion of these cells, in the larger islets, is much less than that of either the alpha or gamma type.

The granules of the beta cells are fairly evenly distributed throughout the cytoplasm. In very thin sections a faintly lighter area beside the nucleus may be apparent in some of the cells. The granules are larger than those of the alpha cells and, occasionally, cells are found in which some of them approach the size of small zymogen granules. If such undoubted islet cells were stained by less specific methods, they might be interpreted as transitional phases of acinar cells. A small proportion of the cells show the granules slightly more numerous and somewhat larger along their outer borders.

The nucleus, which is usually in the center of the cell, is typically spherical, and in most cases has a distinct, oxyphilic nucleolus, placed in the center. This nucleolus is about half the size of that of the acinar cells, the relative size seeming to remain constant. The thickly granular cytoplasm that intimately surrounds it gives the nuclear membrane a sharp outline. On account of the larger granules occasionally seen and the distinct nucleolus in the center of the nucleus the beta cells, except in shape and color, resemble acinar cells more than do those of any other type in the islet.



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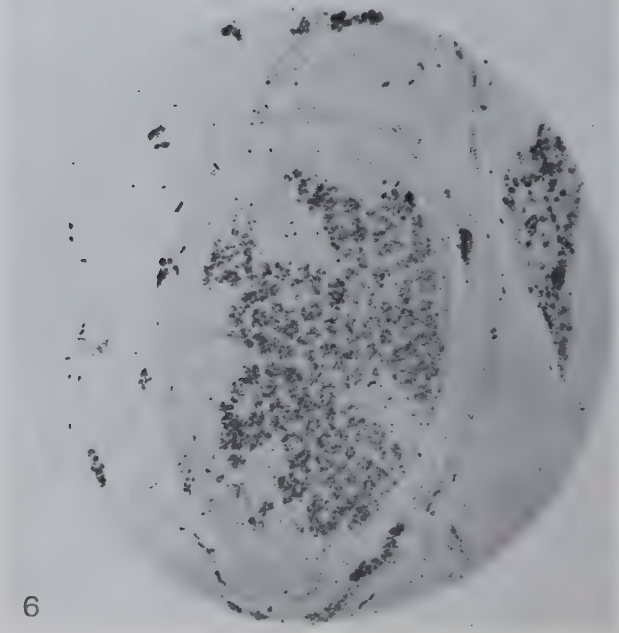

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Gamma cells. The gamma cells are largely responsible for the lighter appearance of the peripheral zone of the islet, and when the technique described above is employed the granules take a bright red color. They may exist in some areas fairly evenly intermixed with beta cells (fig. 5), and in others freely distributed among the alpha cells, or they may be seen to occupy areas of considerable size that are free from both of these types, forming a comparatively loose tissue in which capillaries are not conspicuous. The outlines of the cells are not well defined, excepting where they are bordered by one of the other types. Cells having contact with a capillary are frequently elongated (fig. 5), while others have a more regularly oval shape. They are often larger than the other types of cells and the nucleus is also larger. They are found in the largest as well as the smallest islets and may be present in greater numbers than either or both of the other types.

The granules are comparable in size with those of the beta cells. They are not densely packed in the cytoplasm, but may be slightly increased in number and size in that portion of the cell directed toward, or in contact with, a capillary.

The nucleus is located near the center of the cell in most cases. It is spherical or elliptical in outline and has one or more small nucleoli. It is more vesicular than the nucleus of

Fig. 5 In this photograph the beta cells may be distinguished by their dark-staining granules densely packed throughout the cytoplasm; by their irregular and often pyramidal outlines, and by the comparatively large single nucleolus in the center of the nucleus. The gamma cells appear as light-staining, thinly granular cells, roughly oval in outline and with a nucleus that shows only faintly. The beta and gamma cells are about evenly intermixed, the former tending to surround the latter. In some parts of the upper and lower portions of the photograph the beta and gamma cells alternate to form irregular rosettes (*R*) about blood vessels cut in cross-section. To the left is seen the capsule of the islet, while at the extreme right a small area of faintly outlined alpha cells is shown. Zenker fixation (modified). Aniline acid fuschin, thionin, aurantia (after Kull). × 665.

Fig. 6 This photograph shows the alpha cells occupying about half of the area of one of the islets. The specific technique developed by Lane for alpha cells was employed, namely, alcohol chrome sublimate fixation and staining with neutral gentian. The alpha cells show plainly, while the others in the islet are only very faintly visible. A small area of dark-staining zymogenous cells is seen at the bottom and another at the top of the figure. × 140.

the other types of cells. The nuclear membrane may show distinctly, but in many cells it is only faintly outlined.

In close proximity to the alpha cells there is often seen a small proportion of cells apparently related to the gamma, having a highly vesicular, smoothly elliptical nucleus, with very little cytoplasm, in which few, if any, granules can be distinguished (fig. 2).

The blood supply to the larger islets is by means of small arterioles that are carried in the fibrous capsule and give off branches to penetrate the islet.

While the greater part of the islet tissue occurs in the typical spherical nodular form, one may also find islets composed of only a few cells, closely related to the zymogenous tissue, and with no apparent limiting capsule (fig. 4). In the critical examination of a great number of such combinations with the best apochromatic lenses, we have failed to find any unquestioned examples of transitions between acinar and islet cells. Our findings support the theory that islets are developed, under normal conditions, from the epithelium of the smallest ductules. Large ducts are not often found in the gray snapper, nor have we ever seen any evidence of islet cells originating in such ducts, as has been shown by Jackson to be the case in the pancreas of Elasmobranchs.

The smallest islets are made up mostly of gamma cells, with perhaps a few of the beta type. As the islets grow larger the proportion of beta cells increases for a time, and then the alpha cells begin to appear toward the center. A study of serial sections gives this impression. This fact tends to support the suggestion of Bensley that the gamma cells may be the source of the alpha cells. Contact between alpha and beta cells is rare; the gamma cells practically always intervene between the two types. Since the gamma cells seem to be the first to appear in the islet, this may mean that they are potent to develop into alpha cells on the one hand and beta cells on the other, though sufficient proof of such potentiality is lacking.

SUMMARY AND CONCLUSIONS

1. The islet tissue of a species of Teleostei, the gray snapper —Neomaenis griseus—was treated with special granule stains and found to give reactions similar to those of mammalian islet tissue.

2. Three very distinct types of islet cells were found.

3. A new neutral stain has been developed that offers advantages over other neutral stains previously used.

4. A possible source of alpha and beta cells is suggested.

5. No evidence of transition between acinar and islet cells was seen.

6. A tissue has been described that may prove favorable for further study of islet cells under experimental conditions.

In conclusion I wish to thank Dr. David Fairchild, whose interest in scientific investigations prompted him to facilitate this research in many essential ways. My thanks are also due to N. A. McCormick, M.A., for his zeal in collecting and classifying the material, and for his careful dissection and preservation of the tissues. To Miss V. A. Gillett I am indebted for the photographs. I also wish to thank Prof. J. J. R. Macleod for many valuable suggestions during the progress of the work.

For the sake of comparison with other stains, table 1 is appended.

Adding acetic acid or alcohol to a neutral fixative (the β cell fixative of Lane) reverses the color reaction of the islet cells without changing that of the zymogenous cells. It may be noted that acetic-acid fixatives give good results, though Lane advised against its use.

TABLE 1

FIXATIVE	NEUTRAL GENTIAN	ACID AZO-FUCHSIN BASIC ETHYL VIOLET	ANILIN ACID FUCHSIN METHYL GREEN	EOSIN METHYLENE BLUE	ETHYL VIOLET BIEBRICH SCARLET	EHRLICH'S HEMATOXYLIN AND EOSIN
70 per cent alcohol	α–Violet β–Yellow					
Alcohol chrome sublimate	α–Violet β–Yellow	α–Violet β–Red		α–Blue β–Red		
Bensley's acetic osmic bichromate			α–Deep Red β–Green γ–Clear			
Zenker's (2 per cent acetic acid)	α–Violet β–Yellow	α–Violet β–Red (brick) γ–Red (light)	α–Green β–Dull Red γ–Bright Red	α–Blue β–Brick Red γ–Light Red	α–Blue β–Purple γ–Light Red	α–Dark Red β–Medium Red γ–Light Red
Zenker's (without acetic acid)	α–Yellow β–Violet	α–Red β–Violet			α–Red β–Blue	
Neutral formalin, then acetic osmic bichromate			α–Dull Red β–Green γ–Red			

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